EXHIBIT 1

UNITED STATES DISTRICT COURT
EASTERN DISTRICT OF WISCONSIN
MILWAUKEE DIVISION

SCOTT WEAVER, Individually and
on Behalf of All Others Similarly Situated,

Plaintiff,

v. Case No. 2:18-cv-1996-JPS

CHAMPION PETFOODS USA INC. and
CHAMPION PETFOODS LP,

Defendants.

EXPERT REPORT OF KATERINA MASTOVSKA

I. INTRODUCTION:

I am an Associate Director of Research, Development and Innovation at Eurofins Food Integrity & Innovation (formerly Covance Food Solutions). I was asked to review bisphenol A (BPA) test results in pet food samples tested by Eurofins Food Integrity & Innovation ("Eurofins") on behalf of Greenberg Traurig, LLP representing Champion Petfoods. I have been retained as an expert in this matter for Defendants Champion Petfoods USA Inc. and Champion Petfoods LP's ("Champion").

II. PROFESSIONAL EXPERIENCE AND QUALIFICATIONS:

I am an Associate Director of Research, Development and Innovation at Eurofins Food Integrity & Innovation (formerly Covance Food Solutions), where I direct the development and validation of new analytical methods, and research and adoption of new technologies. I serve as a scientific leader in the community and represent Eurofins at scientific meetings and organizations. Prior to joining Covance in September 2009, I was a Research Chemist with the United States Department of Agriculture's (USDA) Agricultural Research Service (ARS) and served as an expert in the United Nations Food and Agricultural Organization (FAO) panel of the Joint FAO/WHO Meeting on Pesticide Residues (JMPR). I obtained my PhD and master's (summa cum laude) degrees in Food Chemistry and Analysis from the Institute of Chemical Technology (now University of Chemistry and Technology), Faculty of Food and Biochemical Technology, Prague, Czech Republic, in 2002 and 1998, respectively.

Eurofins Food Integrity & Innovation is an accredited, contract testing laboratory and a leader in setting industry standards in food analysis through scientific contributions and leadership roles in standard setting organization, such as AOAC International. AOAC International is a globally recognized, independent, not-for-profit association and voluntary

consensus standards developing organization founded in 1884. When analytical needs arise within a community or industry, AOAC International is the forum for finding appropriate science-based solutions through the development of microbiological and chemical standards, mainly AOAC official methods. AOAC standards are used globally to promote trade and to facilitate public health and safety. Due to my scientific contributions and leadership, I became a Fellow of the AOAC International in 2014. I am a member of the AOAC Official Methods Board (OMB) and a past co-chair of the AOAC Community on Chemical Contaminants and Residues in Food. Among other advisory and expert activities, I have served as an AOAC International Topic Advisor, Study Director, Chair and member of working groups and Expert Review Panels, including an Expert Review Panel on BPA. My scientific work, leadership and expertise have been recognized by multiple AOAC International and other awards.

My scientific and research work has been focused on the analysis of chemical constituents and residues in complex materials, including foods, since 1995. I have developed and validated numerous analytical methods in the area of contaminant and residue testing, and I authored numerous presentations and publications on these topics. In terms of the analysis of BPA, I authored a method for BPA analysis that was approved AOAC First Action Official method 2017.15. I published this method together with a single-laboratory validation study in a peer-reviewed article in the Journal of AOAC International. Also, I was invited to speak on the BPA analysis topic at the North American Chemical Residue Workshop (NACRW) in July 2018 and at the upcoming American Chemical Society (ACS) meeting in August 2019.

A copy of my Curriculum Vitae, which is attached as **Exhibit A,** describes my educational background, experience, qualifications, awards and publications in greater detail.

III. PRIOR TESTIMONY:

During the previous four years, I have testified as a rebuttal witness at trial in the case of *Council for Education and Research on Toxics v. Starbucks Corporation, et al.*, BC435759 (lead case) and *v. Brad Barry Company, Ltd., et al.,* BC461182 (consolidated with lead case), Superior Court in the State of California, County of Los Angeles. I also testified by deposition on May 29, 2019 in the case of *Reitman v. Champion Petfoods*, No. 2:18-CV-01736-DOC in the Central District of California.

IV. COMPENSATION:

I am a regular salaried employee of Eurofins Food Integrity & Innovation and am not compensated separately for my work in this matter. I understand that Eurofins Food Integrity & Innovation charges an hourly rate of $250 for my services in this matter.

V. DOCUMENTS REVIEWED:

In forming the opinions stated herein, I was provided and considered the documents and authorities set forth in **Exhibit B**.

VI. METHODOLOGY:

The following is a brief summary of the methodology used for BPA analysis of the discussed pet food samples. All work was performed in compliance with Eurofins standard operating procedures (SOPs).

A. Sample Receipt and Preparation

Documentation of the samples upon receipt at the site was maintained in the Eurofins Laboratory Information Management System (LIMS). Each sample received a unique sample number. All reporting included both the Eurofins Sample Number and the sponsor's Sample ID Number. Plastic utensils were not used during any sample preparation procedures. Sample processing was documented in the Eurofins LIMS.

B. Sample Analysis

The samples were analyzed for BPA using the Eurofins method for determination of BPA by LC-MS/MS, which is based on the AOAC First Action Official method 2017.15, attached as **Exhibit C**. Water is added to dry samples prior to the extraction step. BPA is extracted from a sample using 1% acetic acid in acetonitrile after addition of a stable-isotope labeled BPA internal standard (BPA-d16). Sodium chloride is used to salt out BPA into the acetonitrile phase. After centrifugation, a freeze-out step is used to remove co-extracted lipids. An aliquot of the supernatant upper layer is then analyzed using high performance liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) in electrospray negative ionization mode.

High specificity of the method is achieved by the use of tandem mass spectrometry (MS/MS) with three precursor-to-product ion MS/MS transitions generated for the BPA molecule. Stable-isotope labeled internal standard (BPA-d16) is used for BPA signal normalization to compensate for any potential volumetric or matrix effects.

BPA is a widely-used monomer for producing polymer materials and also widely distributed in the environment. It can be present in reagents and materials or leach from plastic containers and plastic labware. The Eurofins method procedure was carefully designed and verified to minimize BPA contamination during the sample preparation steps and analysis by avoiding certain materials, such as QuEChERS extraction salts, that contain variable levels of BPA. Moreover, a method (reagent) blank is analyzed with each sample batch and has to contain no more than 50% reporting limit level of BPA (corrected for the nominal sample mass of the sample). Method blank results above this BPA background level disqualifies the run. The BPA level detected in the method blank is subtracted from the level calculated to be present in the samples.

C. Quality Control

Each sample batch was analyzed with method (reagent) blanks and fortified recovery samples as quality control for background levels of BPA and method performance, respectively.

VII. VALIDATION:

The Eurofins method for determination of BPA by LC-MS/MS has been validated in compliance with Eurofins SOPs in a wide range of sample matrices, including infant formula, commercially packaged non-alcoholic beverages, pet food, botanicals, food matrices, and product simulants used in food packaging migration studies, attached as **Exhibit D**. The method was evaluated by AOAC International and approved AOAC First Action Official method 2017.15 in beverages.

For pet food analysis, the method limit of quantification (LOQ) was established at 5 ng/g (5 ppb). This LOQ was validated by analysis of pet food samples fortified at 5 ng/g and analyzed in triplicate on 3 different days (n = 9) by two different analysts with acceptable accuracy (overall mean recovery of 90.1%) and precision (relative standard deviation of 6.1%). **Figure 1** provides representative chromatograms of BPA obtained during the method validation in a method blank (BPA not detected), evaluated pet food matrix and the same pet food matrix spiked with BPA at 5 ng/g.

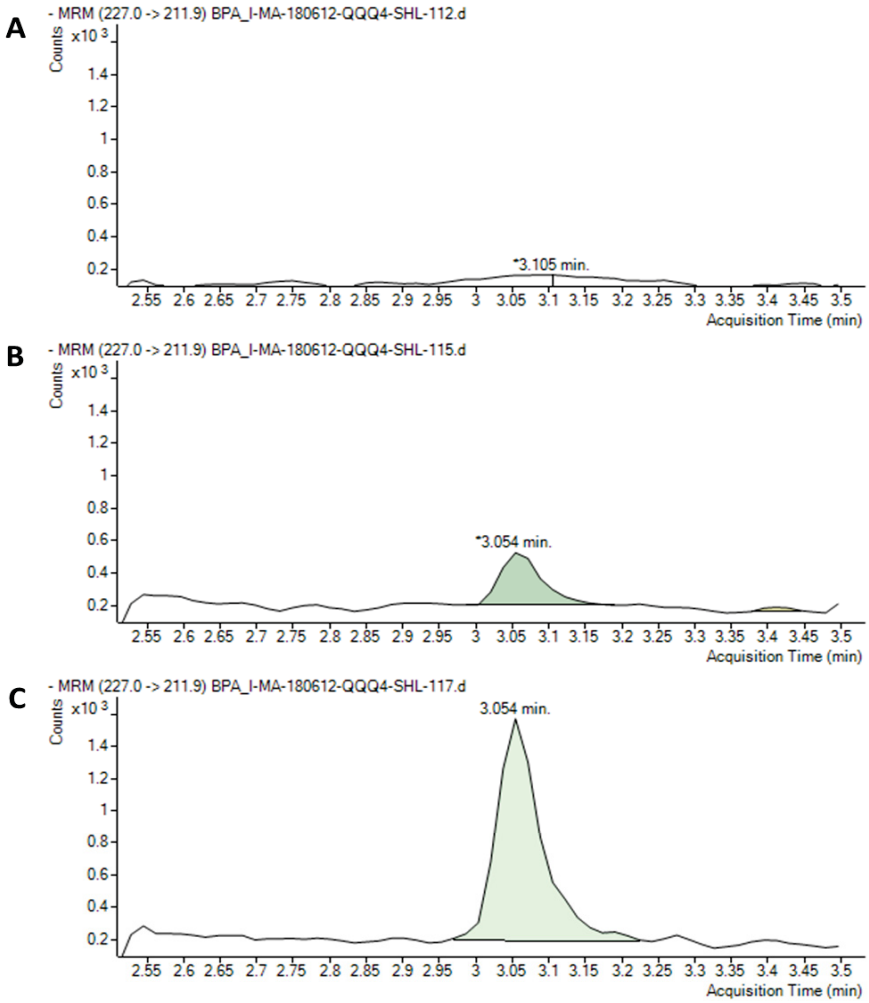

Figure 1. Representative BPA extracted ion chromatograms of (A) method blank, (B) the evaluated pet food sample matrix and (C) the same sample matrix spiked with BPA at 5 ng/g.

VIII. CHAIN OF CUSTODY:

The Champion Petfoods products for analysis were shipped from Champion Petfoods to Eurofins Food Integrity & Innovation in intact, sealed commercial packaging. Chain of custody documentation was initiated at the laboratory for the corresponding samples from each package, as documented in the declaration from Champion Petfoods attached as **Exhibit E.**

The samples for analysis of other pet food brands were received via sealed overnight shipment from Ramboll. Chain of custody documentation initiated in conjunction with the collection of these samples and identifying assigned Sample ID numbers was included with the shipment and maintained by the laboratory thereafter. The assigned sample IDs and descriptions provided did not indicate the brand or specific diet of each sample so that the laboratory was blind to this information at the time of analysis. Subsequently, the brand and specific diet information for each Sample ID has been disclosed, as documented in the declaration from Ramboll attached as **Exhibit F**.

IX. RESULTS AND INTERPRETATION:

Eurofins tested twenty-four (24) Champion dog food product samples and ten (10) blinded dog food samples from other manufacturers. The results of the BPA analysis (expressed on "as is" basis) are summarized in **Table 1** and **Table 2**, respectively, and the Certificates of Analysis are attached as **Exhibit G**.

Table 1. BPA results (concentration in ng/g, or ppb) in the tested Champion dog food product samples:

Eurofins Sample ID	Description	Lot No. or ID Code	Conc. (ng/g)
7318485	DOR4550R-12OZ, ORIJEN Tundra Dog Food	1017750M3	<5.00
7318486	DAC3265-12OZ, ACANA Heritage Meats Dog Food	3007599-80534	<5.00
7318487	DAC3254-12OZ, ACANA Pork and Squash Singles Dog Food	3007122-73454	<5.00
7318488	DOR4440-12OZ, ORIJEN Regional Red Dog Food	3007880-80875	<5.00
7318489	DAC3257-12OZ, ACANA Wild Mackerel Singles Dog Food	3008798-73204	<5.00
7501716	DOR4400-12OZ, ORIJEN Puppy Dog Food	3007509-80454	<5.00
7501717	DOR4430-12OZ, ORIJEN Six Fish Dog Food	3008011-81075	<5.00
7501718	DOR4440-12OZ, ORIJEN Regional Red Dog Food	3007826-80814	<5.00
7501719	DAC3160-12OZ, ACANA Regionals Meadowland Dog Food	3007642-80465	5.90
7501720	DAC3190-12OZ, ACANA Regionals Appalachian Ranch Dog Food	3007252-80195	<5.00
7501721	DAC3261-12OZ, ACANA Heritage Free-Run Poultry Dog Food	3008164-81274	5.30
7501722	DAC3263-12OZ, ACANA Heritage Freshwater Fish Dog Food	3008150-81245	19.6
7501723	DAC3251-12OZ, ACANA Singles Lamb & Apple Dog Food	3007310-80255	<5.00
7501724	DAC3255-12OZ, ACANA Singles Duck & Pear Dog Food	3007398-80305	<5.00

7501725	DAC3257-12OZ, ACANA Singles Mackerel & Greens Dog Food	3007133-80045	<5.00
7747568	ORIJEN Regional Red Dog Food	1018928M3	<5.00
7747569	ORIJEN Tundra Freeze Dried Dog Food	1019167T3	<5.00
7747570	ORIJEN Adult Freeze Dried Dog Food	1018689T3	<5.00
7747571	ORIJEN Six Fish Dog Food	1018435M3	<5.00
7747572	ORIJEN Puppy Dog Food	1018809M3	<5.00
8657388	ORIJEN Original Dog Food	3011808-91284	<5.00
8657389	ORIJEN Puppy Large Breed Dog Food	3011646-91151	<5.00
8657390	ACANA Grasslands Dog Food	3011204-90804	5.43
8657391	ACANA Wild Atlantic Dog Food	3011800-91274	<5.00

Table 2. BPA results (concentration in ng/g, or ppb) in the blinded competitor dog food samples:

Eurofins Sample ID	**Description**	**Lot No. or ID Code**	**Conc. (ng/g)**
8212745	Fromm Adult, Chicken and Brown Rice dog food	030119-D27	<5.00
8212746	Farmina, N&D Fish and Orange dog food	030119-D26	<5.00
8212747	Ziwi USA, New Zealand Tripe and Lamb freeze dried dry dog food	030119-D29	<5.00
8212748	Now Fresh, Grain-Free, Fish Adult dry dog food	030119-D28	<5.00
8212749	Instinct, Original Grain Free, Beef dog food	030119-D2	9.12
8212750	Taste of the Wild, Pacific Stream dog food	030119-D13	10.7
8212751	Open Farm, Surf & Turf freeze dried dog food	030119-D23	<5.00
8212752	GO! Fit and Free, Grain Free, Chicken, Turkey, Trout dog food	030119-D1	<5.00
8212753	Merrick, Back Country Raw Infused, Pacific Catch, Salmon, Whitefish, and Trout dog food	030119-D7	8.01
8212755	Instinct, Original Grain Free, Beef dog food (duplicate)	030119-D30	9.46

The results in the tested Champion Petfoods products ranged from <5.00 to 19.6 ng/g. The results in the blinded samples from other manufacturers ranged from <5.00 to 10.7 ng/g.

All quality control samples (method blanks and fortified recovery samples) analyzed in sample batches together with the test samples met the acceptance criteria stated in the method.

Table 3 summarizes recovery results for the fortified recovery QC samples spiked at 10 ng/g. Acceptable recoveries in the range of 80.9 – 109% were obtained. The recoveries were determined as marginal % recovery after subtraction of BPA concentration in the unspiked sample from the concentration determined in the spiked sample and divided by the added concentration.

Table 3. Fortified recovery QC results:

Spike Sample ID	Unspiked Sample ID	Recovery (%)
7395331	7318485	90.6
7395334	7318486	109
7395335	7318488	102
7395336	7318489	89.3
7502235	7501719	91.3
7502229	7501727	92.1
7502237	7501733	90.0
7792771	7747576	88.9
8272204	8212745	94.5
8272205	8212746	94.7
8272208	8212747	87.9
8272209	8212748	85.0
8272211	8212749	92.0
8272212	8212750	109
8272213	8212751	99.0
8272214	8212752	85.6
8272215	8212753	96.1
8272216	8212755	97.4
8665500	8657388	96.5
8665501	8657389	84.1
8665502	8657390	89.0
8665503	8657391	80.9

X. SUMMARY OF OPINIONS:

Based upon my review, Eurofins tested the discussed pet food samples using a scientifically valid and properly validated method applicable to BPA analysis in the tested pet food samples. The quality control and data acceptance criteria of the method were met. The reported results are reliable with regard to the identification of BPA and with regard to the quantification of BPA in the samples. The results in the tested Champion Petfoods products ranged from <5.00 to 19.6 ng/g (<5.00 to 19.6 ppb). The results in the blinded samples from other manufacturers ranged from <5.00 to 10.7 ng/g (<5.00 to 10.7 ppb).

Executed on this 3rd day of August, 2019.

Katerina Mastovska, PhD

EXHIBIT A

CURRICULUM VITAE: KATERINA MASTOVSKA

CURRENT POSITION

Title:	Associate Director - Research, Development and Innovation
Department:	Eurofins Food Integrity and Innovation (EFII) Madison, WI, USA

EDUCATION

PhD Food Chemistry and Analysis, Institute of Chemical Technology (ICT), Faculty of Food and Biochemical Technology, Prague, Czech Republic, January 2002

MS Food Chemistry and Analysis (summa cum laude), Institute of Chemical Technology (ICT), Faculty of Food and Biochemical Technology, Prague, Czech Republic, June 1998

PROFESSIONAL EXPERIENCE

2016 - present: Associate Director - Research, Development and Innovation, Eurofins Food Integrity and Innovation (formerly Covance Food Solutions), Madison, WI, USA

Dr. Mastovska leads the global chemistry R&D and Innovation group at Eurofins Food Integrity and Innovation (EFII), which was formerly Covance Food Solutions (CFS). She directs the internal development of new analytical methods, and research and adoption of new technologies to support strategic business growth objectives. Dr. Mastovska serves as a scientific leader in the community and represents EFII at scientific meetings and organizations. She is a member of the EFII senior leadership team responsible for setting out the vision for the organization and operational and scientific plans.

2013-2016: Associate Scientific Director, NCFS, Covance Laboratories Inc., Madison, WI, USA
2011-2013: Lead Staff Scientist, NCFS, Covance Laboratories Inc., Madison, WI, USA
2009-2011: Senior Technical Manager, NCFS, Covance Laboratories Inc., Greenfield, IN, USA

Dr. Mastovska was responsible for development of new analytical methods and strategies for testing of chemical residues, contaminants, and adulterants in food and dietary supplements. She served as a scientific and technical resource for the Nutritional Chemistry and Food Safety (NCFS) department in this field. In addition to leading new analytical method development, she also took a lead role in identifying and solving analytical problems, and in interfacing with clients in terms of technical expertise, problem solving, and business development activities.

2002-2009: Research Chemist, USDA, Agricultural Research Service, Wyndmoor, PA, USA

Dr. Mastovska developed efficient methods for pesticide and veterinary drug residues and other chemical contaminants in food, such as acrylamide or dioxins, mainly based on advanced gas and liquid chromatographic techniques coupled with (tandem) mass spectrometry. She investigated and evaluated new analytical techniques and tools to be implemented in chemical residue analysis, and conducted successful method transfer to routine testing laboratories.

1995-2002: Researcher, Laboratory of Food Contaminants & Toxicants, ICT, Prague, Czech Republic

Dr. Mastovska worked during her undergraduate, graduate and post-graduate studies as a researcher developing and running methods for analysis of pesticide residues and other food and environmental contaminants, primarily based on gas chromatographic separations with mass spectrometric or element-selective detection. She participated in numerous international projects, mainly focused on method harmonization within the EU. She spent three short-term stays as a visiting scientist in Pesticide Residue Group at the Central Science Laboratory (CSL – now FERA) in York, UK in 1997, 1998, and 1999 and one two-month stay at the USDA Agricultural Research Service in Wyndmoor, PA in 2000, conducting successful method development for the analysis of pesticide residues.

EXPERT AND ADVISORY ACTIVITIES

AOAC International
Official Methods Board (OMB) member (2015 – present)
Co-chair of the AOAC Int. Community on Chemical Contaminants and Residues in Food (2011 – 2016)
Chair of the Expert Review Panel on MCPD and glycidyl esters (2017 – present)
Chair of the Furans Working Group (2019 – present)
Chair of the PDE5 Inhibitor Working Group for the Stakeholder Panel on Dietary Supplements (2014)
Working group member: Allergens by mass spectrometry (2016), BPA in beverages (2017), MCPD and GE esters (2017), Cannabinoids in cannabis plant and concentrates (2016), Cannabinoids in chocolate and pesticide residues in cannabis (2017-2018), Sugars (2018)
Dietary Supplement Expert Review Panel on PDE5 inhibitors, member (2015 – present)
Bisphenol A Expert Review Panel, member (2017 – present)
Cannabinoids Expert Review Panel, member (2017 – present)
Study Director for the AOAC Int. study on polycyclic aromatic hydrocarbons (PAHs) in seafood as a response to the oil spill in the Gulf of Mexico (2010 – 2013)
AOAC Int. Topic Advisor for the Veterinary Drug Residue Methods (2009 – present)
Veterinary Drug Expert Review Panels, member (2009 – present)

Joint FAO/WHO Meeting on Pesticide Residues (JMPR)
Dr. Mastovska served (2006-2009) as an expert in the UN Food and Agricultural Organization (FAO) panel of the JMPR (Joint FAO/WHO Meeting on Pesticide Residues) evaluating pesticide submissions and recommending world-wide pesticide maximum residue levels in foods and feeds to the Codex Alimentarius Commission.

Other activities:
International Symposium on Recent Advances in Food Analysis (RAFA) – scientific committee member (2017-present)
European Commission, Research Executive Agency (REA) – independent expert evaluating research proposals (2010-present)
North American Chemical Residue Workshop (NACRW) – program committee and organizing committee member (2010-present)
International Symposium on Recent Advances in Food Analysis (RAFA) – instructor of interactive seminars (2011, 2013, 2015, 2017)
Interagency Residue Control Group (IRCG) – member (2006-2009)

USDA FSIS Surveillance Advisory Team (SAT) – member (2006-2009)
General Mills, Medallion Laboratories, Minneapolis, MN – consultant (2006-2009)
International Atomic Energy Agency (IAEA) – invited advisor (invitation declined in 2009)
Georgian National Science Foundation – invited grant reviewer (2006-present)
Residue Analytical Workshop at ICT, Prague – invited lecturer (2006, 2009)
USDA/EPA Pesticide Workshop for FIFRA laboratories – co-organizer and instructor (2004)
Peer-reviewer for: J. AOAC Int., J. Chromatogr. A, Anal. Chim. Acta, Anal. Chem., J. Agric. Food. Chem, J. Anal. Bioanal. Chem., Food Chem., Food Addit. Contam., Talanta, Metabolomics, *etc.*

PROFESSIONAL MEMBERSHIPS AND AFFILIATIONS

American Chemical Society, Divisions of Agrochemicals and Analytical Chemistry
American Society for Mass Spectrometry
AOAC International

HONORS AND AWARDS

AOAC Int. Expert Review Panel of the Year award, 2018
AOAC Int. Award for Achievement in Technical and Scientific Excellence, 2017
AOAC Int. Method of the Year award, 2016 (AOAC 2015.12 method)
AOAC Int. Award for Achievement in Technical and Scientific Excellence, 2016
AOAC Int. Method of the Year award, 2015 (AOAC 2014.08 method)
AOAC Int. Fellow award, 2014
Finalist of Covance inaugural Science and Technology award, 2014
AOAC Int. Study Director of the Year award, 2013
AOAC Int. Expert Review Panel of the Year award, 2013
Scientific Program Chair of the 50th Florida Pesticide Residue Workshop/North American Chemical Residue Workshop (FPRW/NACRW), 2013
ARS Technology Transfer Award for "Partners in QuEChERS", Outstanding Effort, 2009
Federal Laboratory Consortium (FLC) Mid-Atlantic Regional Excellence in Technology Transfer Award, 2009
Excellence in Government Award for private sector involvement, Silver Medalist, Federal Executive Board, 2008
U.S. Department of Agriculture Certificate of Merit for outstanding research contributions, 2006, 2007, 2008, and 2009
U.S. Department of Agriculture Extra Effort Award, 2005
Granted U.S. Permanent Residency in the Extraordinary Ability category, 2005 (Naturalized U.S. Citizen since August 2011)
U.S. Environmental Protection Agency Certificate of Appreciation, 2004
Josef Hlavka Award for the best young researchers in the Czech Republic who demonstrated exceptional abilities and creative thinking in their field, 1998
ICT Rector Award, 1998

CURRICULUM VITAE: KATERINA MASTOVSKA

PUBLICATIONS

Peer-Reviewed Articles:

L. Vaclavik, F. Benes, M. Fenclova, J. Hricko, A. Krmela, V. Svobodova, J. Hajslova, K. Mastovska: Quantitation of cannabinoids in *Cannabis* dried plant materials, concentrates and oils using liquid chromatography–diode array detection technique with optional mass spectrometric detection: A single-laboratory validation study. First Action 2018.11. *J. AOAC Int.* (in press).

S. Li, J. Shippar, K. Mastovska: Determination of bisphenol A (BPA) in commercially packaged ready-to-consume carbonated and non-carbonated water and non-alcoholic beverages: A single-laboratory validation study. First Action 2017.15. *J. AOAC Int.* **102** (2019) 605-611.

U. Koesukwiwat, L. Vaclavik, K. Mastovska: Method development and validation for total haloxyfop analysis in infant formulas and related ingredient matrices using liquid chromatography-tandem mass spectrometry. *Anal. Bioanal. Chem.* **410** (2018) 5521-5528.

L. Vaclavik, J. Shippar, U. Koesukwiwat, K. Mastovska: Method development and validation for low-level propineb and propylenethiourea analysis in baby food, infant formula, and related matrices using liquid chromatography-tandem mass spectrometry. *Food Addit. Contam.* **35** (2018) 2387-2399.

H. Zhao, J. Zulkoski, K. Mastovska: Development and validation of a multi-class, multi-residue method for veterinary drug analysis in infant formula and related ingredients using UHPLC-MS/MS. *J. Agric. Food Chem.* **65** (2017) 7268-7287.

L. Vaclavik, J.R. Schmitz, J.-F. Halbardier, K. Mastovska: Single-laboratory validation study of a method for screening and identification of phosphodiesterase type 5 inhibitors in dietary ingredients and supplements using liquid chromatography/quadrupole–orbital ion trap mass spectrometry: First Action 2015.12. *J. AOAC Int.* **99** (2016) 55–72.

K. Mastovska, W.J. Sorenson, J. Hajslova: Determination of polycyclic aromatic hydrocarbons (PAHs) in seafood using gas chromatography-mass spectrometry: Collaborative study, *J. AOAC Int.* **98** (2015) 477-505.

Z. Veprikova, M. Zachariasova, Z. Dzuman, A. Zachariasova, M. Fenclova, P. Slavikova, M. Vaclavikova, K. Mastovska, D. Hengst, J. Hajslova: Mycotoxins in plant-based dietary supplements: Hidden health risk for consumers, *J. Agric. Food Chem.* **63** (2015) 6633-6643.

K. Mastovska: 50th Anniversary of the Florida Pesticide Residue Workshop and the birth of the North American Chemical Residue Workshop, *J. Agric. Food Chem.* **62** (2014) 3649-3650.

S.J. Lehotay, K. Mastovska, A.R. Lightfield, A. Nunez, T. Dutko, C. Ng, L. Bluhm: Rapid analysis of aminoglycoside antibiotics in bovine tissues using disposable pipette extraction and ultrahigh performance liquid chromatography - tandem mass spectrometry. *J. Chromatogr. A* **1313** (2013) 103-112.

K. Mastovska, P.L. Wylie: Evaluation of a new column backflushing set-up in gas chromatographic-tandem mass spectrometric analysis of pesticide residues in dietary supplements, *J. Chromatogr. A* **1265** (2012) 155-164.

S.J. Lehotay, A.R. Lightfield, L. Geis-Asteggiante, M.J. Schneider, T. Dutko, C. Ng, L. Bluhm, K. Mastovska: Development and validation of a streamlined method designed to detect residues of 62 veterinary drugs in bovine kidney using ultrahigh performance liquid chromatography – tandem mass spectrometry. *Drug Testing and Analysis* **4** (2012) 75-90.

M.J. Schneider, K. Mastovska, M.B. Solomon: Distribution of penicillin G residues in culled dairy cow muscle. *J. Agric. Food Chem.* **58** (2010) 5408-5413.

K. Mastovska, K. Dorweiler, S.J. Lehotay, J. Wegscheid, K. Szpylka: Pesticide multiresidue analysis in cereal grains using modified QuEChERS method combined with automated direct sample introduction GC-TOFMS and UPLC-MS/MS techniques, *J. Agric. Food Chem.* **58** (2010) 5959-5972.

U. Koesukwiwat, S.J. Lehotay, K. Mastovska, K. Dorweiler, N. Leepipatpiboon: Evaluation of a modified QuEChERS method for pesticide residues in flaxseeds, peanuts, and doughs, *J. Agric. Food Chem.* **58** (2010) 5950-5958.

S.J. Lehotay, K. Mastovska, A.R. Lightfield, R.A. Gates: Multi-analyst, multi-matrix performance of the QuEChERS approach for pesticide residues in foods and feeds using LC-MS/MS analysis with different calibration techniques. *J. AOAC Int.* **93** (2010) 355-367.

E. Hoh, S.J. Lehotay, K.C. Pangallo, K. Mastovska, H. Ngo, C.M. Reddy, W. Vetter: Simultaneous quantitation of multiple classes of organohalogen compounds in fish oils with direct sample introduction-comprehensive two-dimensional gas chromatography and time of flight mass spectrometry. *J. Agric. Food Chem.* **57** (2009) 2653-2660.

E. Hoh, S.J. Lehotay, K. Mastovska, H. Ngo, W. Vetter, K.C. Pangallo, C.M. Reddy: Capabilities of direct sample introduction - comprehensive two-dimensional gas chromatography – time-of-flight mass spectrometry to analyze organic chemicals of interest in fish oils. *Environ. Sci. Technol.* **43** (2009) 3240-3247.

M.J. Schneider, K. Mastovska, S.J. Lehotay, A.R. Lightfield, B. Kinsella, C. Shultz: Comparison of screening methods for antibiotics in beef kidney juice and serum. *Anal. Chim. Acta* **637** (2009) 290-297.

B. Kinsella, S.J. Lehotay, K. Mastovska, A.R. Lightfield, M. Danaher, A. Furey: New method for the analysis of flukicides and other anthelmintics in bovine milk and liver using liquid chromatography-tandem mass spectrometry. *Anal. Chim. Acta* **637** (2009) 196-207.

S.J. Lehotay, K. Mastovska, A. Amirav, A.B. Fialkov, T. Alon, P. Martos, A. de Kok, A.R. Fernandez-Alba: Identification and confirmation of chemical residues by chromatography-mass spectrometry and other techniques. *Trends in Anal. Chem.* **27** (2008) 1070-1090.

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EXHIBIT B

Documents and Authorities Considered for Expert Report of Dr. Katerina Mastovska

(1) LC-MS/MS analysis results in Agilent MassHunter software – Attached are summaries of obtained peak areas, concentrations (in ng/mL) calculated in the sample extracts (used to calculate BPA concentration in ng/g in the samples as per the method SOP calculation procedure), calibration curves, and BPA chromatograms for the following samples batches:
- BPA_I-MA-180614-1 (samples 7318485 – 7318489)
- BPA_I-MA-180719-2 (samples 7501716 – 7501725)
- BPA_I-MA-181022-1 (samples 7747568 – 7747572)
- BPA_I-MA-190322-1 (samples 8212745 – 8212753, 8212755)
- BPA_I-MA-190724-QQQ5-LK-1 (samples 8657388 - 8657391)

(2) Eurofins SOP for Determination of BPA by LC-MS/MS (previous version in Covance format and current version in Eurofins format) as "**Exhibit C**"

(3) Eurofins (Covance Food Solution) method validation report for determination of bisphenol A (BPA) by LC-MS/MS, attached as "**Exhibit D**":
- Initial method validation report
- Method validation report addendum for pet food analysis

(4) Declaration of Gayan Hettiarachchi containing a table de-blinding Champion dog foods' sample numbers and discussing chain of custody, attached as "**Exhibit E**."

(5) Ramboll Declaration containing a table de-blinding competitor dog foods' sample numbers and discussing chain of custody, attached as "**Exhibit F**."

(6) Eurofins certificates of analysis for the samples in question, attached as "**Exhibit G**."

(7) Expert Report of Sean Callan in case of Reitman v. Champion Petfoods, No. 2:18-CV01736-DOC in the Central District of California.

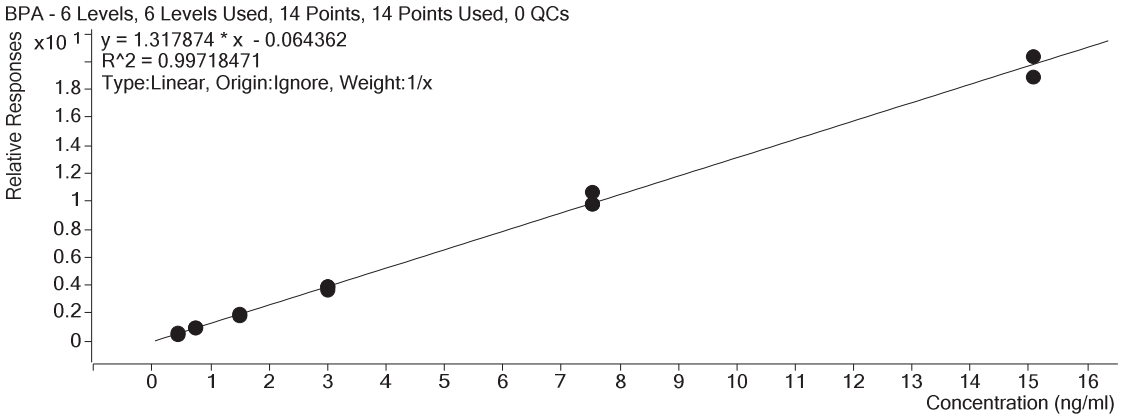
Batch: **BPA_I-MA-180614-1**

Summary Table

		BPA			BPA-d16 (ISTD)
Sample Name	**Type**	**Exp. Conc. (ng/mL)**	**Area**	**Calc. Conc. (ng/mL)**	**Area**
BPA_WS-180612-01	Cal	0.4519	1217	0.3942	2675
BPA_WS-180612-02	Cal	0.7532	2364	0.793	2410
BPA_WS-180612-03	Cal	1.5065	4740	1.5355	2419
BPA_WS-180612-04	Cal	3.0129	9284	2.973	2409
BPA_WS-180612-05	Cal	7.5323	23373	7.472	2389
BPA_WS-180612-06	Cal	15.065	49306	14.4071	2606
Method Blank1	Sample		0	0	3273
Method Blank2	Sample		0	0	3247
7318485_Rep1	Sample		1478	0.2831	4786
7318485_Rep2	Sample		1298	0.2694	4467
7318485_Rep3	Sample		1766	0.3712	4157
7318485 SPK10ppb	Sample		9342	1.6539	4417
Solvent Blank	Blank		0	0	70
7318486_Rep1	Sample		1448	0.3064	4268
7318486_Rep2	Sample		1214	0.2705	4157
7318486_Rep3	Sample		1334	0.2749	4478
7318486 SPK10ppb	Sample		9585	1.8332	4076
BPA_WS-180612-04	Cal	3.0129	9624	2.8015	2653
7318487_Rep1	Sample		1358	0.3147	3876
7318487_Rep1	Sample		1185	0.2634	4190
7318487_Rep1	Sample		1716	0.3584	4206
7318487 SPK5ppb	Sample		5449	0.9885	4400
7318487 SPK5ppb	Sample		5282	0.9907	4256
7318487 SPK5ppb	Sample		5137	0.9927	4129
7318488_Rep1	Sample		2047	0.4112	4287
7318488_Rep2	Sample		2613	0.5597	3881
7318488_Rep3	Sample		2072	0.3927	4572
7318488 SPK10ppb	Sample		11147	1.9826	4374
Solvent Blank	Blank		0	0	30
BPA_WS-180612-05	Cal	7.5323	24444	8.1453	2291
Solvent Blank	Blank		0	0	42
7318489_Rep1	Sample		1027	0.2278	4354
7318489_Rep2	Sample		939	0.2248	4047
7318489_Rep3	Sample		899	0.2062	4336
7318489 SPK10ppb	Sample		7336	1.5263	3767
BPA_WS-180612-01	Cal	0.4519	1492	0.511	2449
BPA_WS-180612-02	Cal	0.7532	2380	0.7815	2465
BPA_WS-180612-03	Cal	1.5065	4484	1.3917	2534
BPA_WS-180612-04	Cal	3.0129	9450	2.9925	2436
BPA_WS-180612-05	Cal	7.5323	23785	7.5047	2421
BPA_WS-180612-06	Cal	15.065	49877	15.4859	2452

Batch: **BPA_I-MA-180614-1**

Calibration curve



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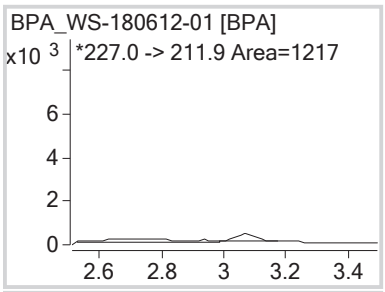

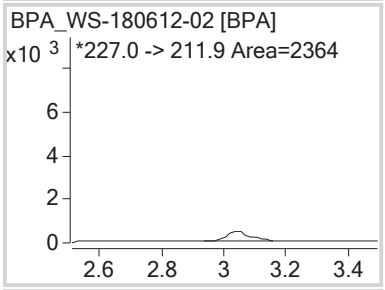

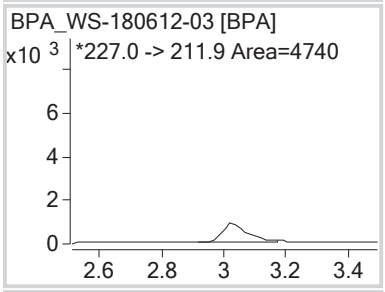

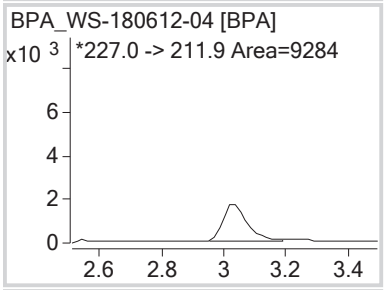

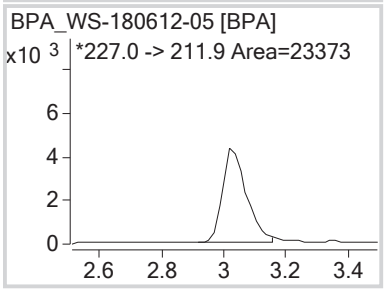

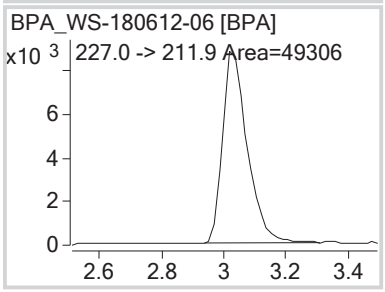

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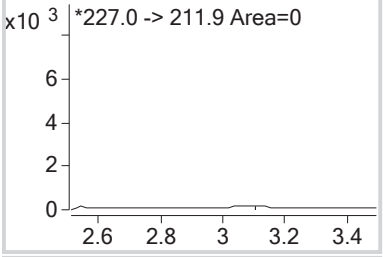

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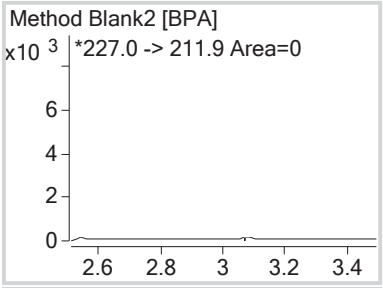

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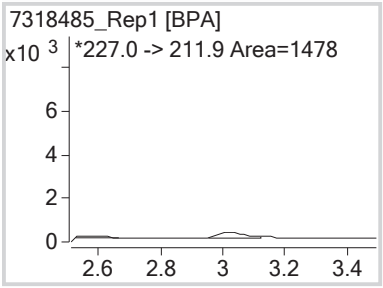

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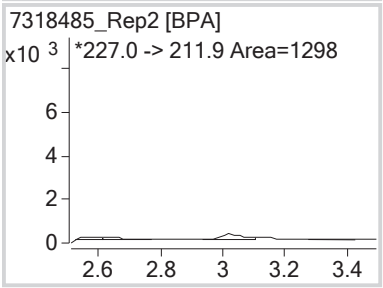

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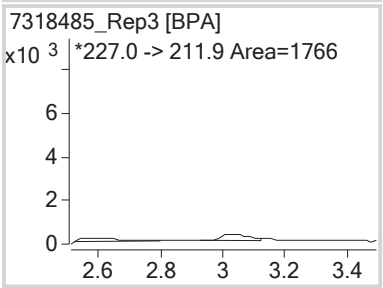

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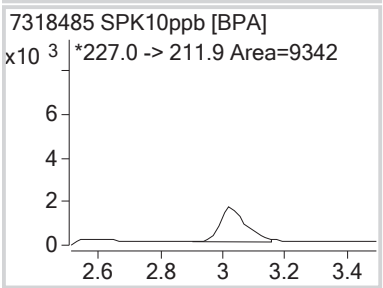

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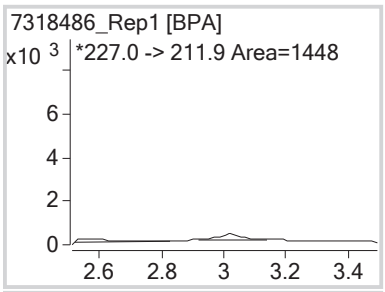

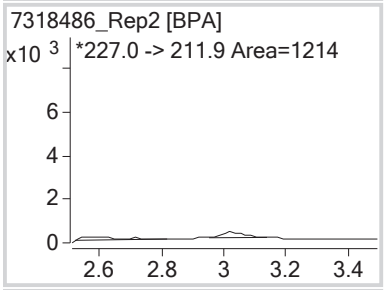

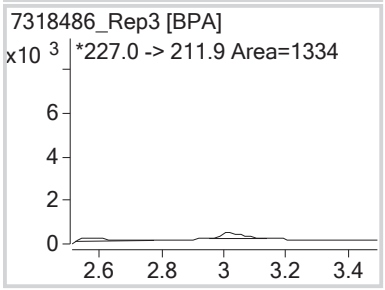

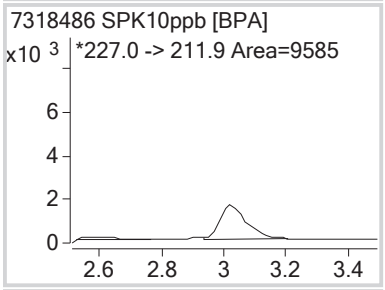

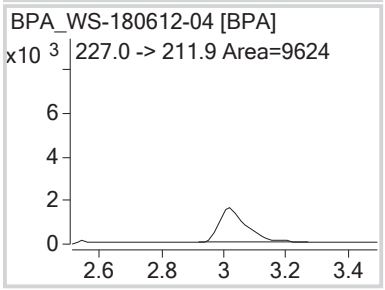

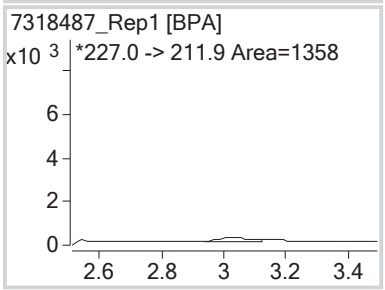

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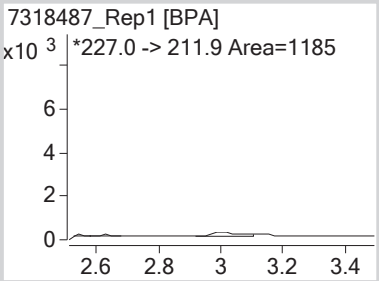

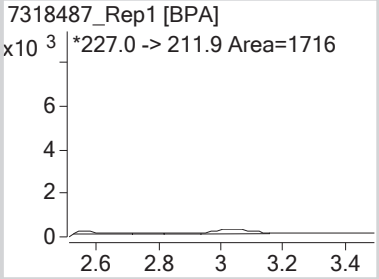

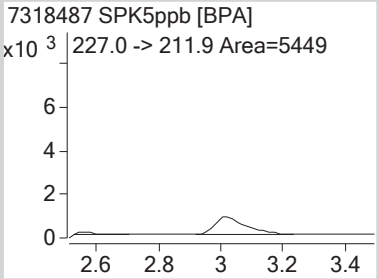

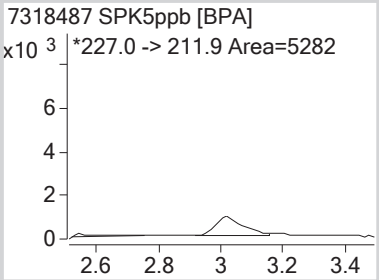

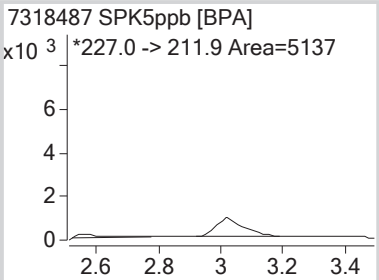

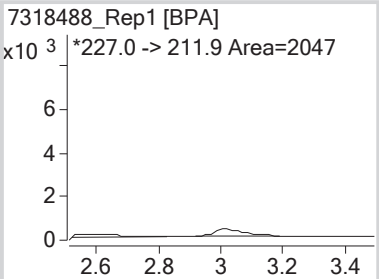

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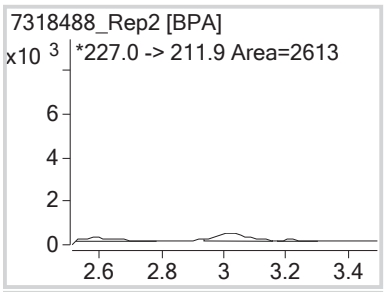

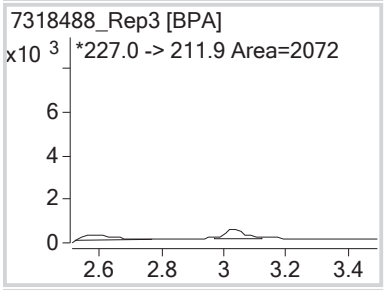

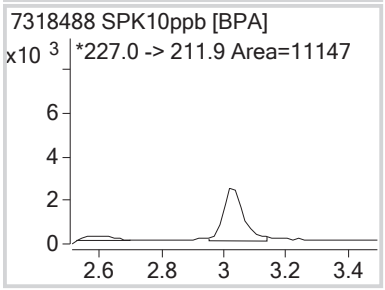

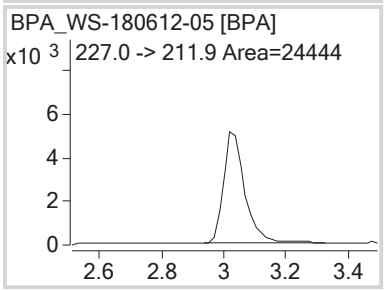

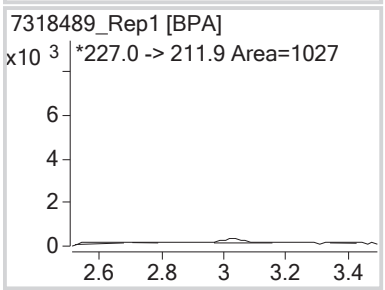

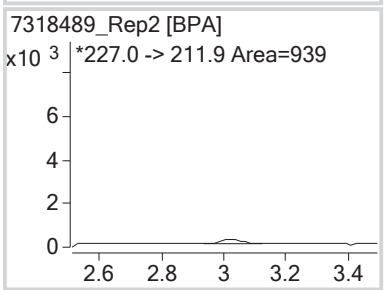

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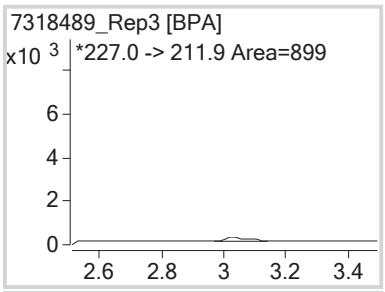
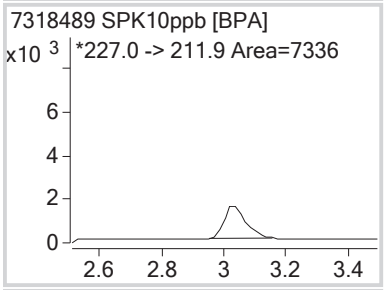
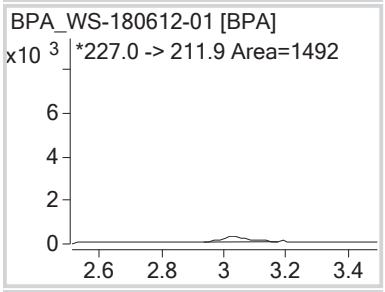
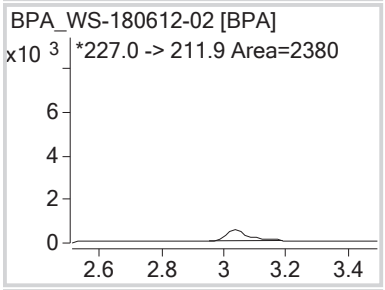
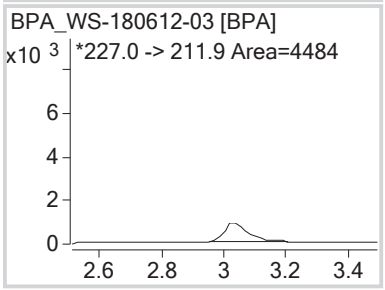
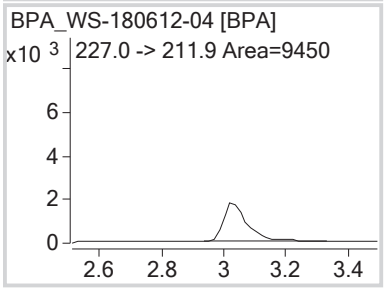


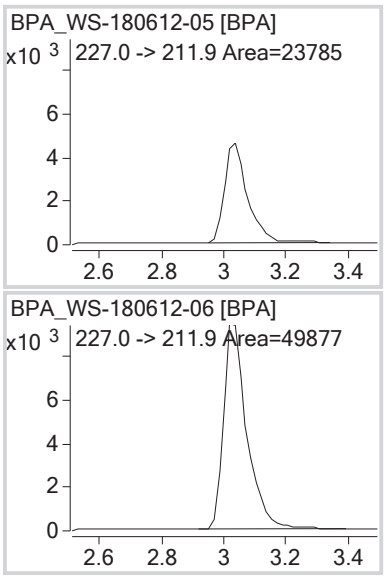











BPA_WS-180612-05 [BPA]
x10 3
227.0 -> 211.9 Area=23785
6
4
2
0
2.6
2.8
3
3.2
3.4

BPA_WS-180612-06 [BPA]
x10 3
227.0 -> 211.9 Area=49877
6
4
2
0
2.6
2.8
3
3.2
3.4

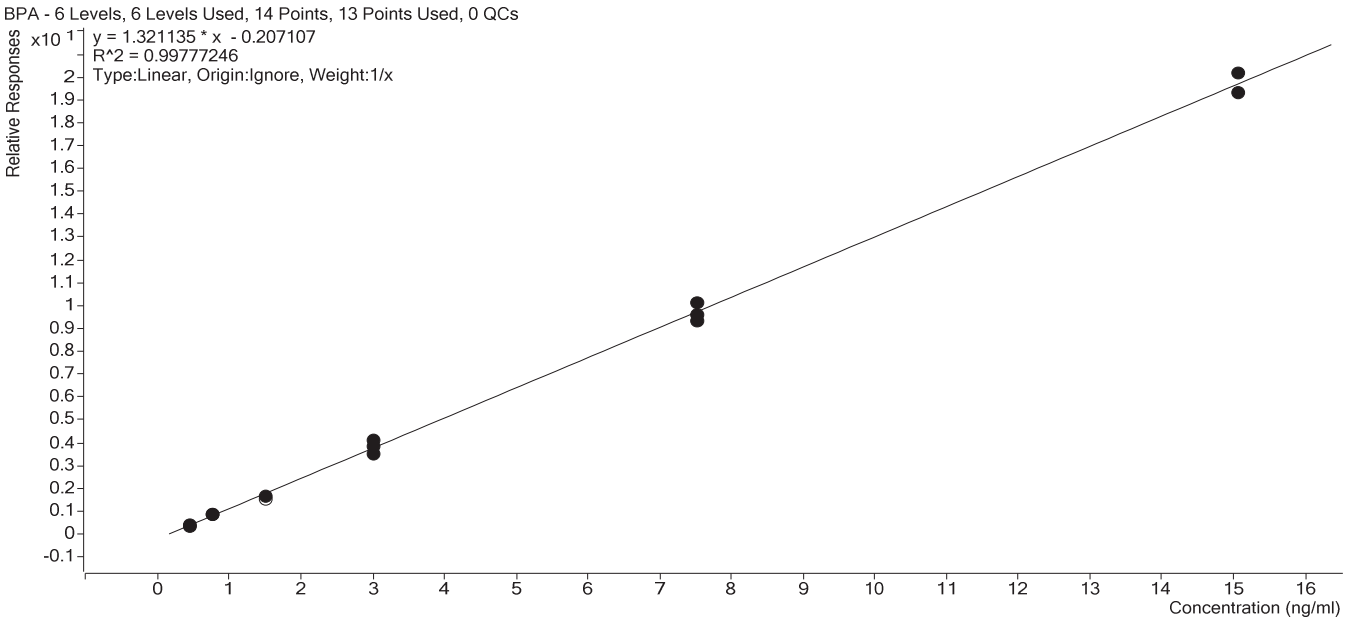
Batch: **BPA_I-MA-180719-2**

Summary Table

		BPA			BPA-d16 (ISTD)
Sample Name	**Type**	**Exp. Conc. (ng/mL)**	**Area**	**Calc. Conc. (ng/mL)**	**Area**
BPA_WS-180719-07	Cal	0.4519	838	0.4429	2216
BPA_WS-180719-08	Cal	0.7532	1743	0.8186	1994
BPA_WS-180719-09	Cal	1.5065	3539	1.3918	2169
BPA_WS-180719-10	Cal	3.0129	7328	2.7889	2107
BPA_WS-180719-11	Cal	7.5323	18822	7.1989	2023
BPA_WS-180719-12	Cal	15.065	43132	15.4278	2138
Method Blank1	Sample		0	0	2580
Method Blank2	Sample		0	0	2519
7501716	Sample		0	0	2571
7501717	Sample		613	0.3328	2636
7501718	Sample		465	0.289	2664
7501719 Rep1	Sample		2408	0.9064	2431
7501719 Rep2	Blank		2430	0.9378	2355
7501719 SPK10ppb	Sample		7086	2.2883	2516
7501720	Sample		799	0.389	2604
7501721	Sample		2413	0.818	2762
7501722	Sample		10437	2.9063	2873
7501723	Sample		188	0.2084	2754
BPA_WS-180719-10	Cal	3.0129	7704	3.0694	2002
7501724	Sample		0	0	2579
7501725	Sample		527	0.3155	2514
7501727	Sample		2238	0.8346	2499
7501727 SPK10ppb	Sample		6639	2.2126	2444
BPA_WS-180719-11	Cal	7.5323	21352	7.8185	2109
7501733 Rep1	Sample		209	0.2137	2780
7501733 Rep2	Sample		148	0.1979	2715
7501733 SPK10ppb	Sample		4887	1.5674	2622
BPA_WS-180719-07	Cal	0.4519	715	0.4081	2153
BPA_WS-180719-08	Cal	0.7532	1911	0.8206	2180
BPA_WS-180719-09	Cal	1.5065	3004	1.2959	1996
BPA_WS-180719-10	Cal	3.0129	8316	3.2746	2019
BPA_WS-180719-11	Cal	7.5323	20387	7.4235	2124
BPA_WS-180719-12	Cal	15.065	45200	14.7989	2337

Batch: **BPA_I-MA-180719-2**

Calibration curve



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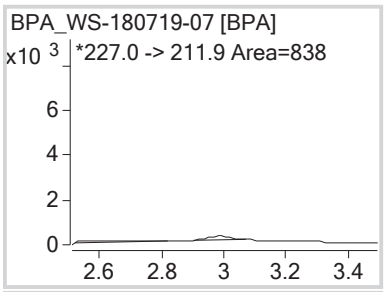

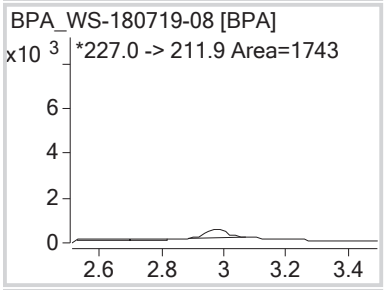

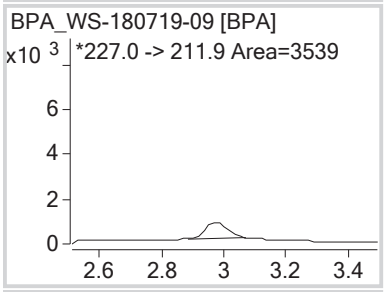

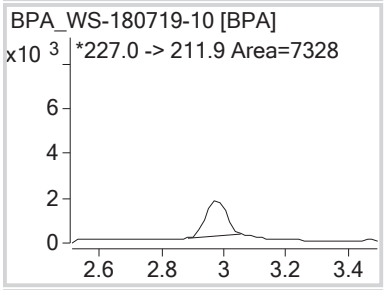

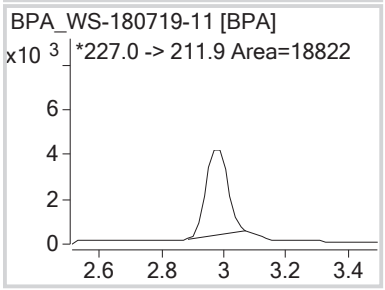

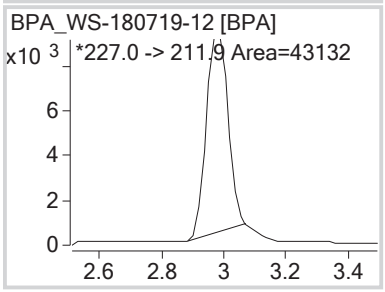

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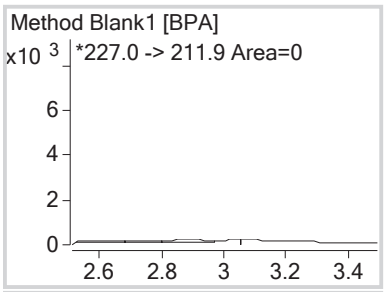

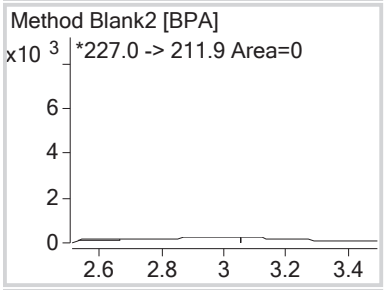

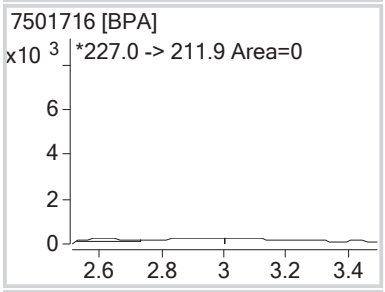

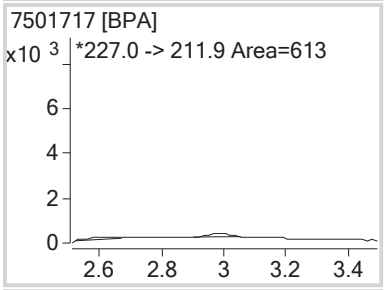

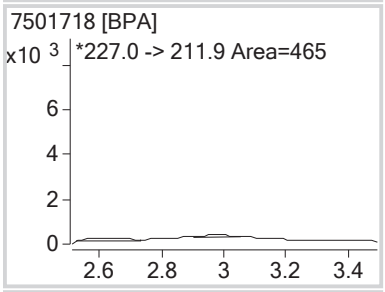

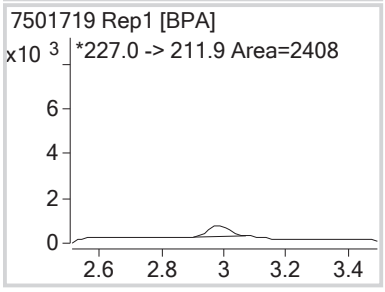

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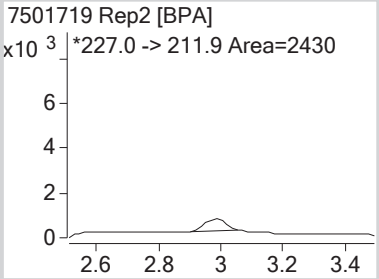

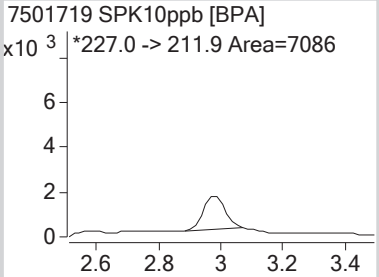

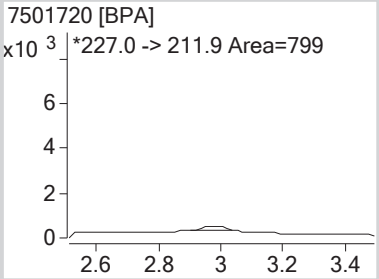

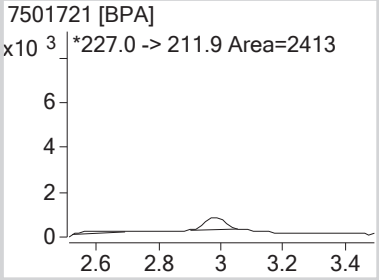

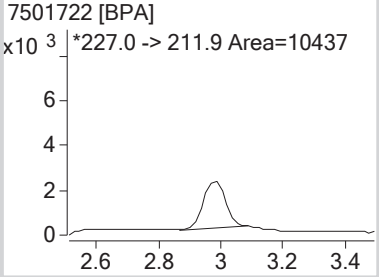

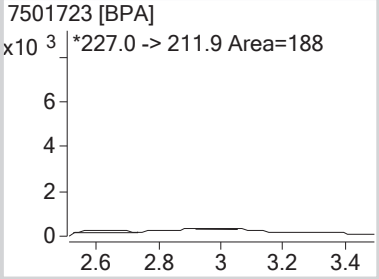

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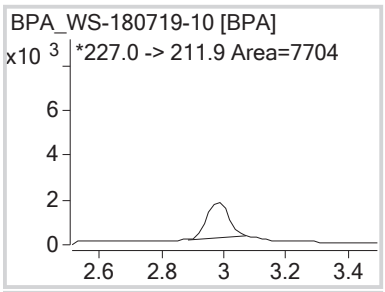

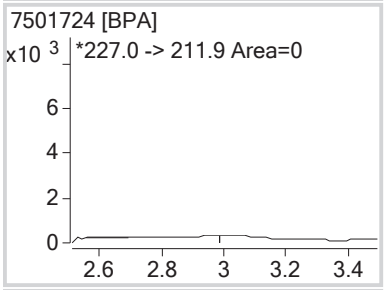

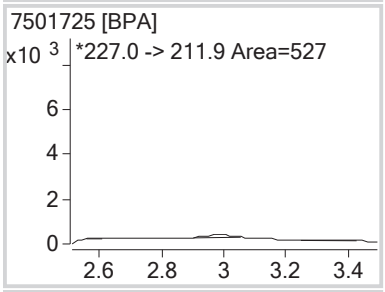

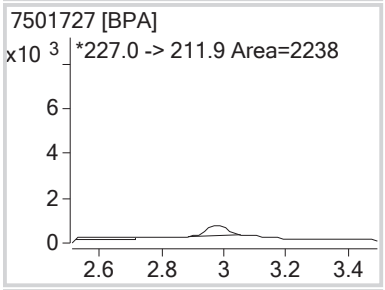

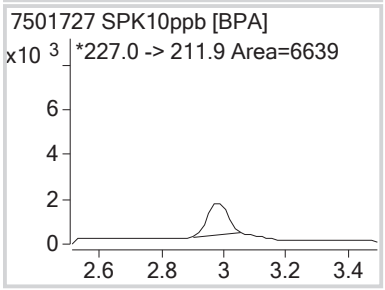

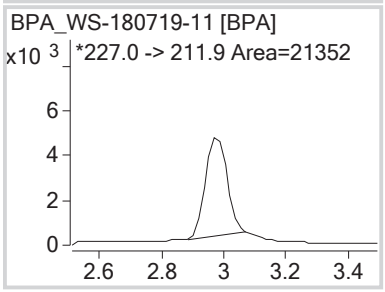

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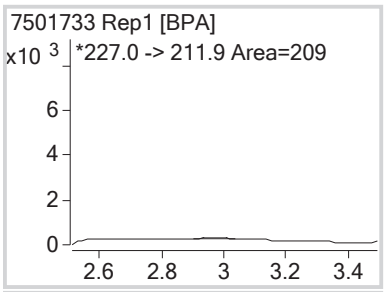

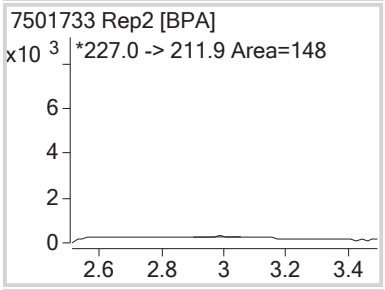

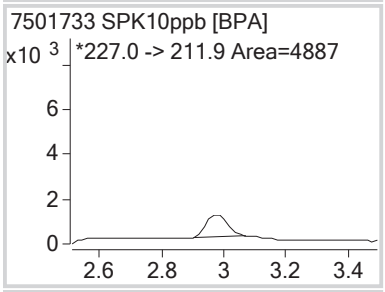

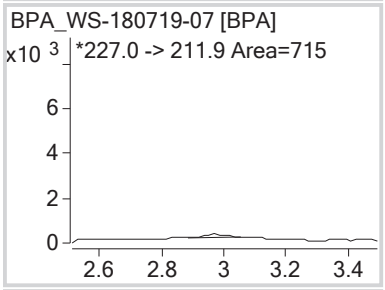

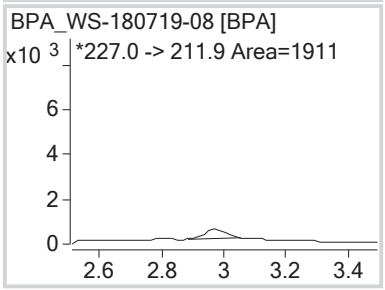

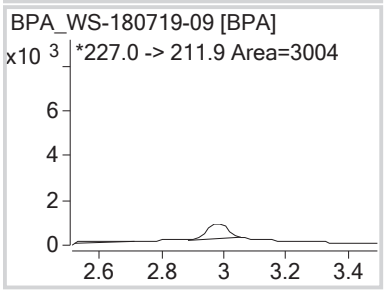

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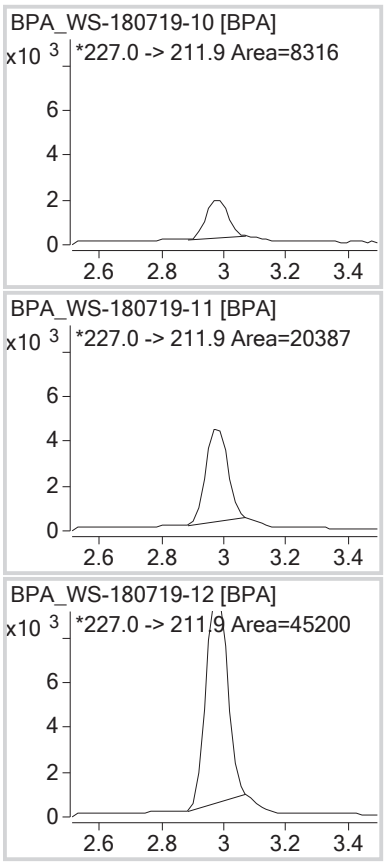


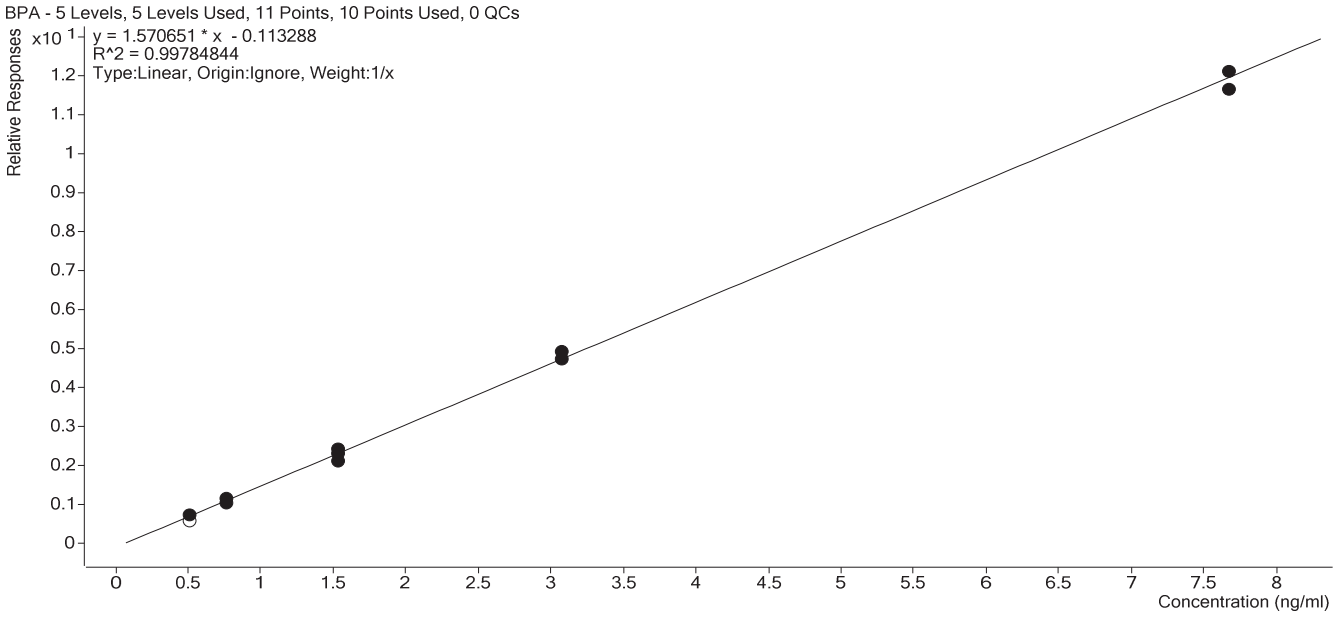
Batch: **BPA_I-MA-181022-1**

Summary Table

		BPA			BPA-d16 (ISTD)
Sample Name	**Type**	**Exp. Conc. (ng/mL)**	**Area**	**Calc. Conc. (ng/mL)**	**Area**
BPA_WS-181023-01	Cal	0.5115	5738	0.4335	10110
BPA_WS-181023-02	Cal	0.7673	10138	0.7226	9923
BPA_WS-181023-03	Cal	1.5346	22740	1.6145	9387
BPA_WS-181023-04	Cal	3.0692	45011	3.2062	9144
BPA_WS-181023-05	Cal	7.6729	111633	7.7745	9228
blank 1	Sample		0	0	12204
blank 2	Sample		0	0	12011
7747568	Sample		9478	0.5785	11917
7747569	Sample		0	0	6329
7747570	Sample		0	0	8822
7747571	Sample		2237	0.1824	12919
7747572	Sample		11928	0.7092	11920
BPA_WS-181023-03	Cal	1.5346	20971	1.5339	9134
7747576	Sample		2004	0.1696	13090
7747576 rec	Sample		114750	5.6255	13156
BPA_WS-181023-01	Cal	0.5115	6642	0.5252	9333
BPA_WS-181023-02	Cal	0.7673	9997	0.7889	8881
BPA_WS-181023-03	Cal	1.5346	19388	1.4017	9284
BPA_WS-181023-04	Cal	3.0692	41389	3.0863	8743
BPA_WS-181023-05	Cal	7.6729	106050	7.4804	9114

Batch: **BPA_I-MA-181022-1**

Calibration Curve



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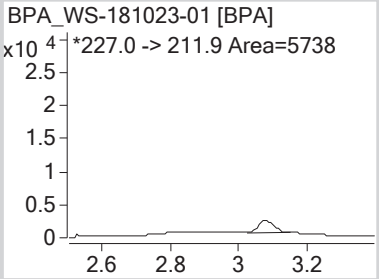

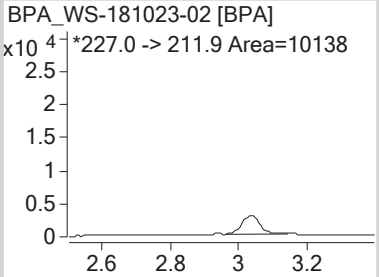

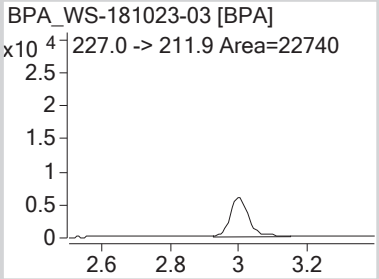

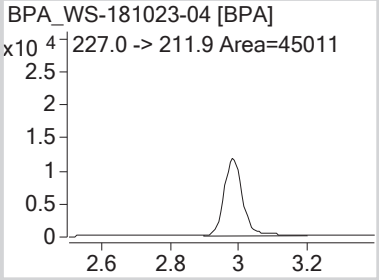

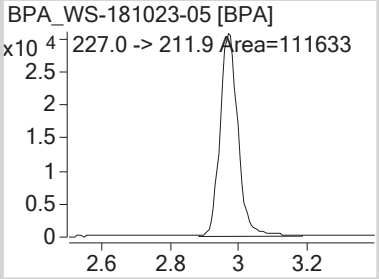

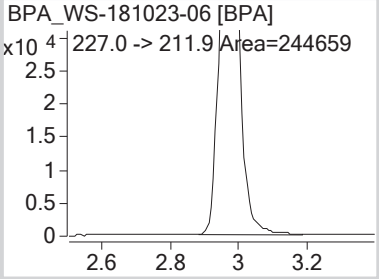

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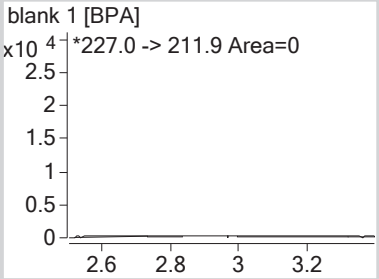

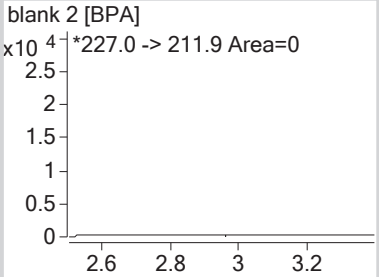

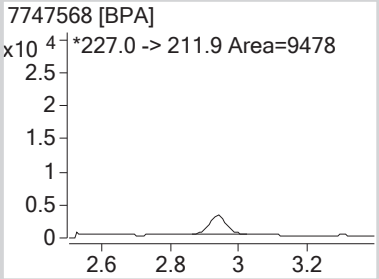

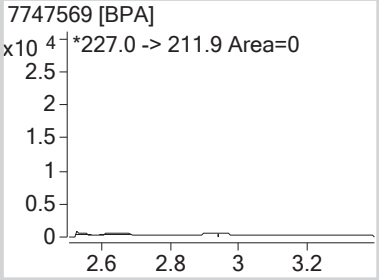

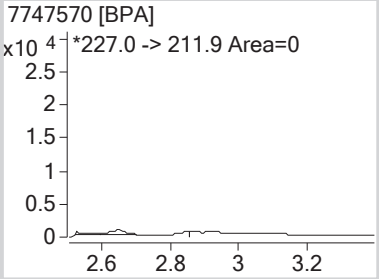

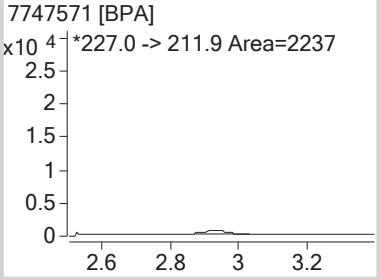

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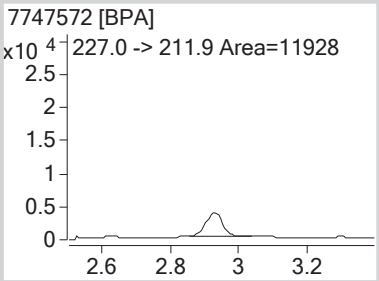

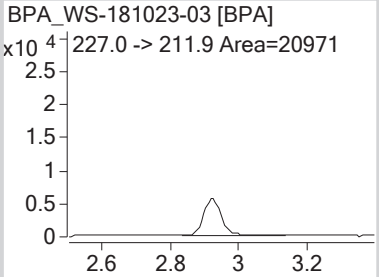

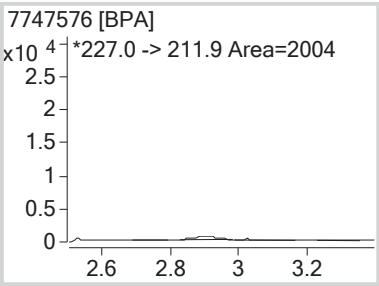

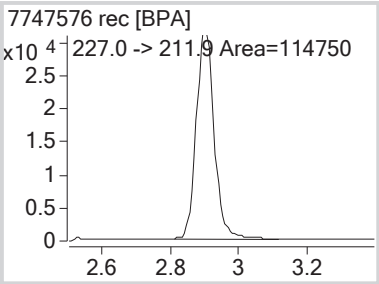

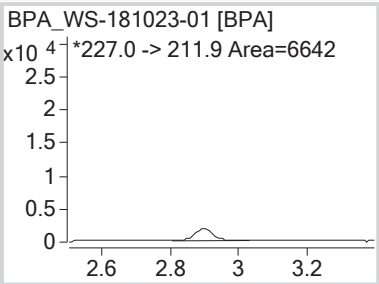

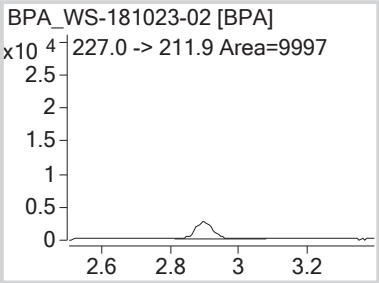

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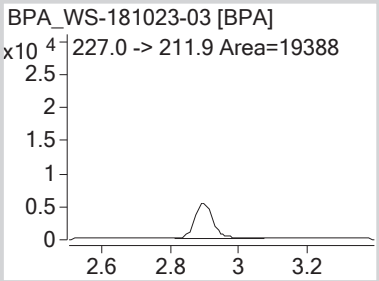
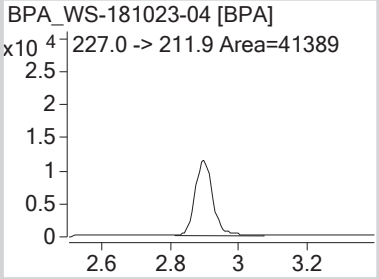
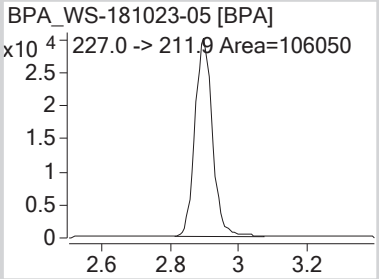
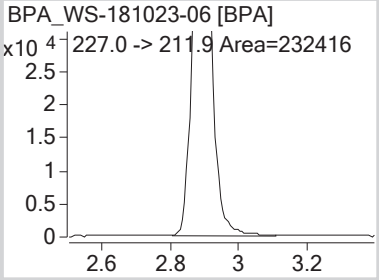








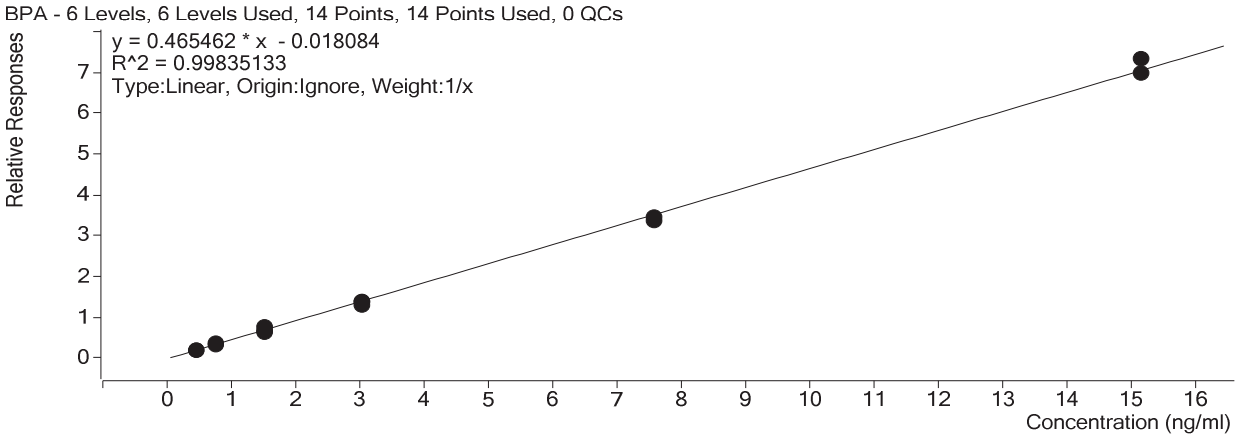
Batch: **BPA_I-MA-190322-1**

Summary Table

		BPA			BPA-d16 (ISTD)
Sample Name	**Type**	**Exp. Conc. (ng/mL)**	**Area**	**Calc. Conc. (ng/mL)**	**Area**
BPA_WS-190322-01	Cal	0.4544	1190	0.4546	6147
BPA_WS-190322-02	Cal	0.7574	2062	0.7748	6020
BPA_WS-190322-03	Cal	1.5147	3832	1.4316	5912
BPA_WS-190322-04	Cal	3.0294	8383	2.9716	6141
BPA_WS-190322-05	Cal	7.5735	23140	7.4964	6666
BPA_WS-190322-06	Cal	15.147	46179	15.7777	6304
blank 1	Sample		0	0	8508
blank 2	Sample		0	0	8236
8212745	Sample		0	0	10465
8212745 rec	Sample		6288	1.4306	9706
8212746	Sample		1981	0.4017	11729
8212746 rec	Sample		10559	1.8379	12610
8212747	Sample		1516	0.2689	14161
8212747 rec	Sample		8124	1.6029	11159
8212748	Sample		1324	0.2871	11456
8212748 rec	Sample		8183	1.5739	11452
BPA_WS-190322-04	Cal	3.0294	10635	3.0002	7715
8212749	Sample		7064	1.4057	11103
8212749 rec	Sample		15797	2.7854	12357
8212750	Sample		8187	1.6271	11075
8212750 rec	Sample		15757	3.3076	10357
8212751	Sample		0	0	10508
8212751 rec	Sample		6752	1.4992	9934
8212752	Sample		1292	0.2792	11548
8212752 rec	Sample		8655	1.5773	12087
8212753	Sample		5819	1.2115	10661
8212753 rec	Sample		14060	2.6795	11439
BPA_WS-190322-03	Cal	1.5147	5836	1.6469	7797
8212755	Sample		8624	1.4458	13170
8212755 rec	Sample		17328	2.9301	12876
8212756	Sample		0	0	10717
BPA_WS-190322-01	Cal	0.4544	1690	0.4789	8251
BPA_WS-190322-02	Cal	0.7574	2668	0.7476	8088
BPA_WS-190322-03	Cal	1.5147	5551	1.5265	8017
BPA_WS-190322-04	Cal	3.0294	10942	2.8364	8403
BPA_WS-190322-05	Cal	7.5735	28294	7.2805	8394
BPA_WS-190322-06	Cal	15.147	58794	15.0733	8402

Batch: **BPA_I-MA-190322-1**

Calibration curve



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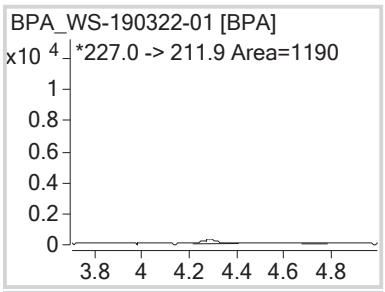

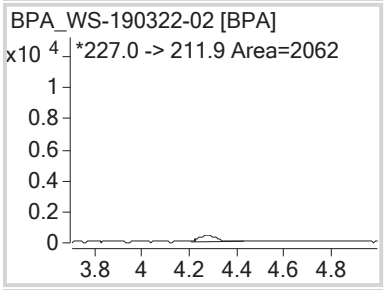

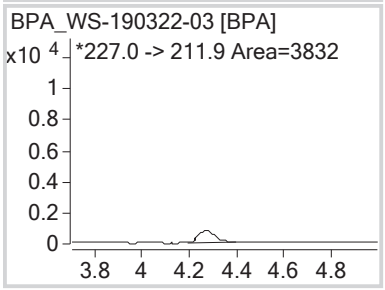

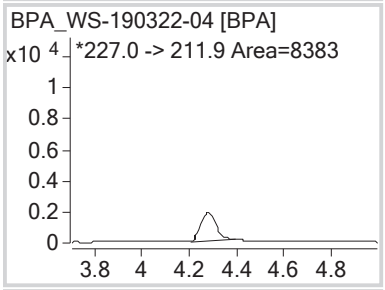

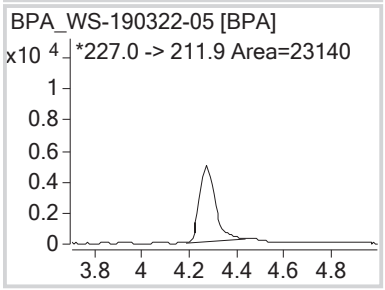

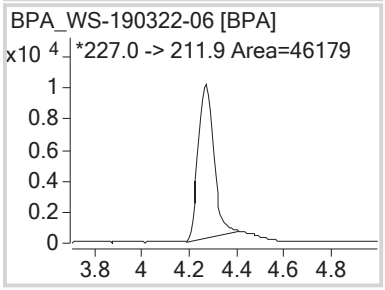

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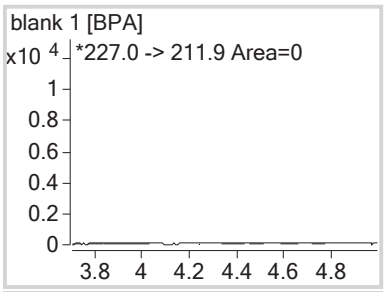

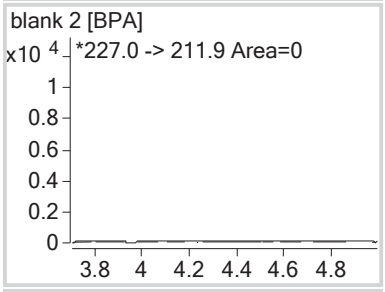

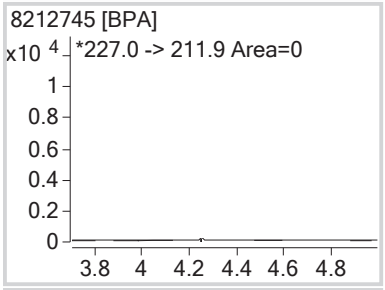

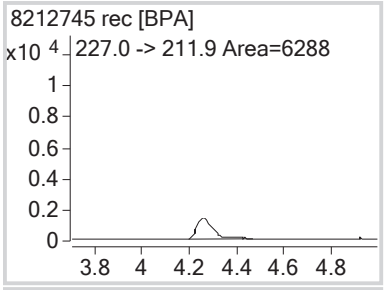

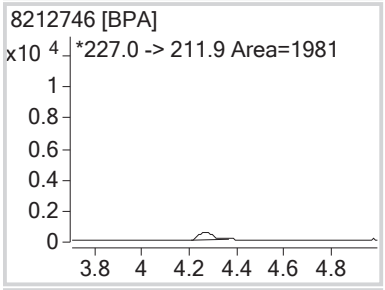

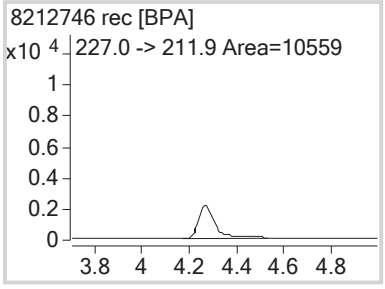

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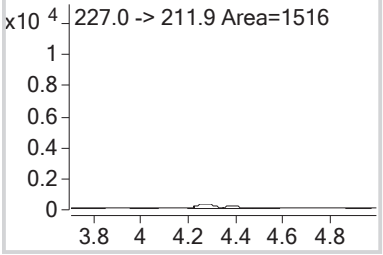
8212747 [BPA]



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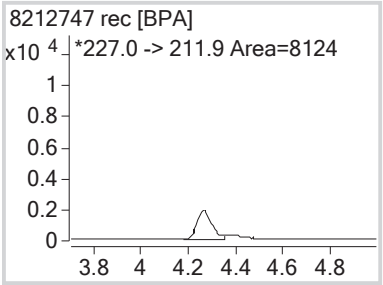

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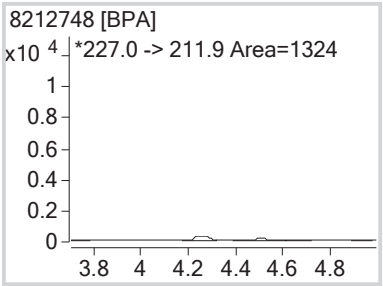

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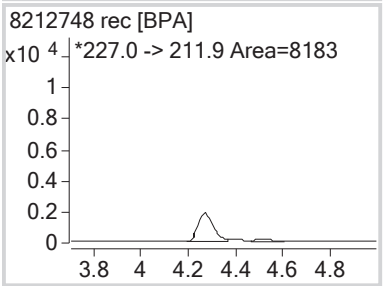
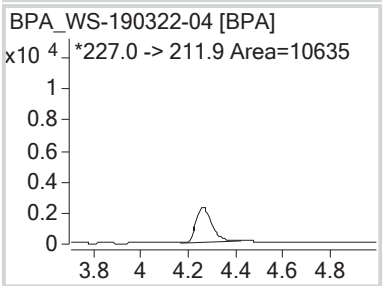


BPA_WS-190322-04 [BPA]



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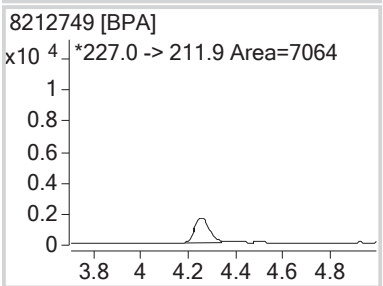

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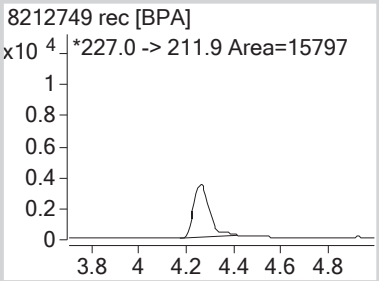

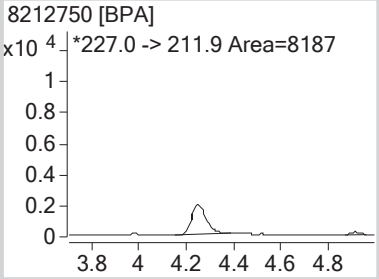

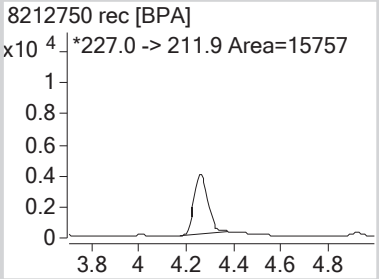

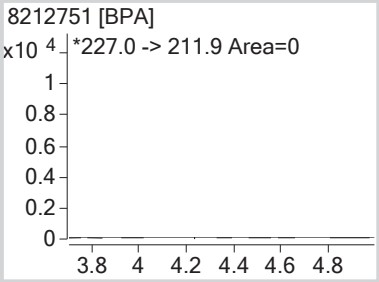

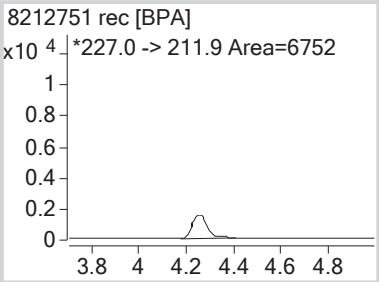

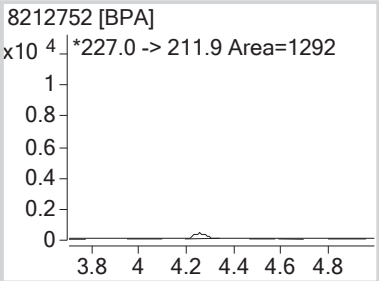

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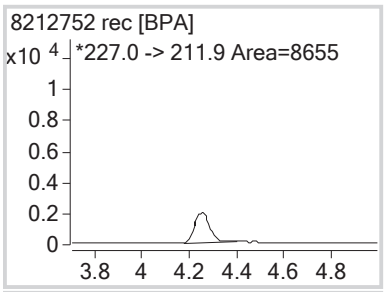

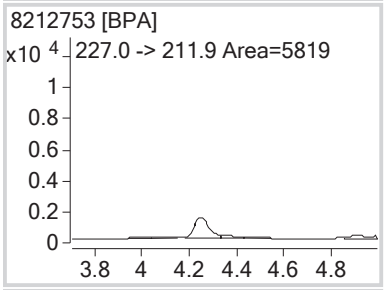

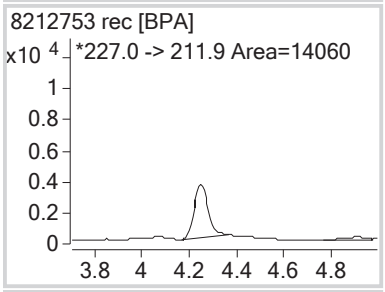

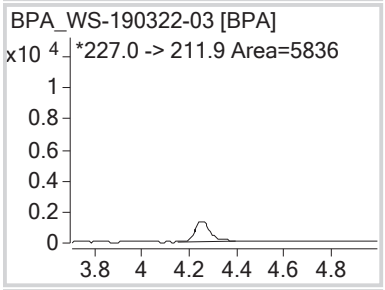

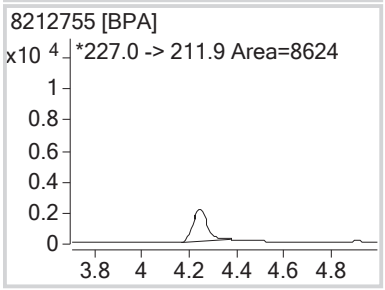

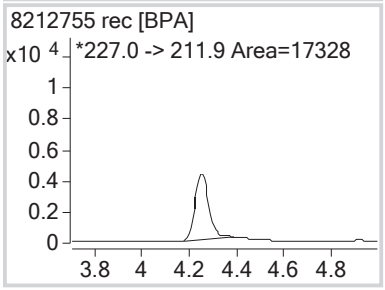

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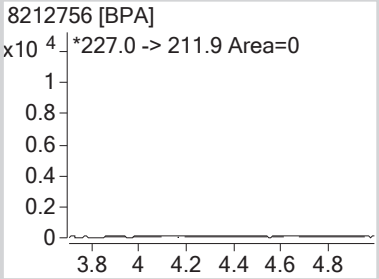

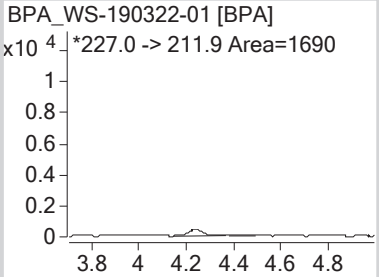

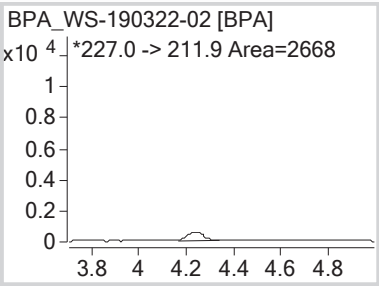

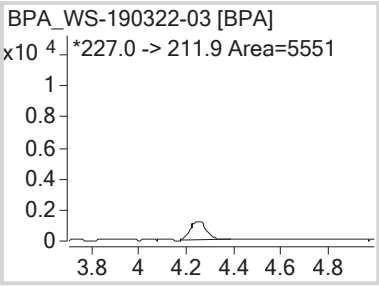

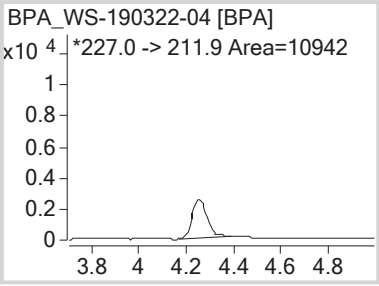

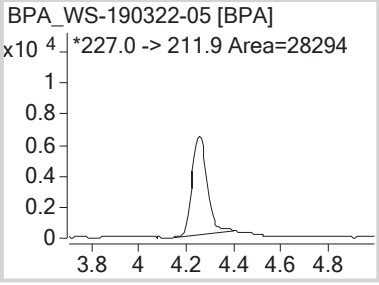

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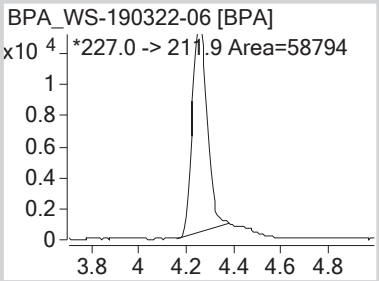


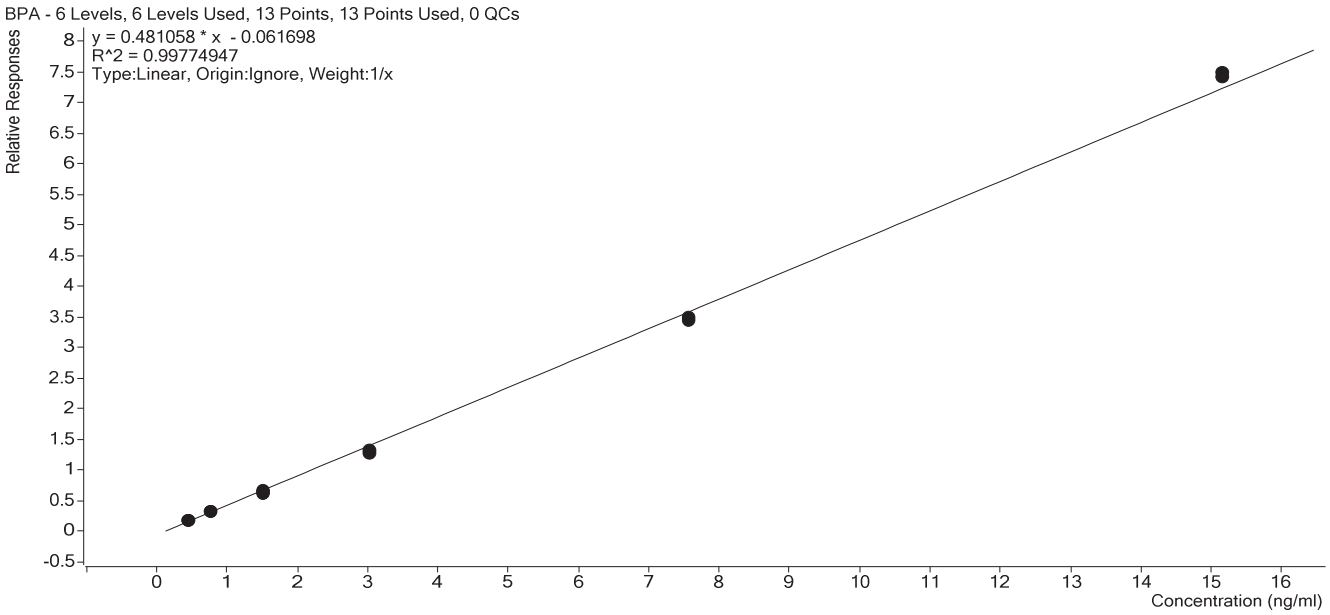
Batch: **BPA_I-MA-190724-QQQ5-LK-1**

Summary Table

		BPA			BPA-d16 (ISTD)
Sample Name	**Type**	**Exp. Conc. (ng/mL)**	**Area**	**Calc. Conc. (ng/mL)**	**Area**
BPA_WS-190724-07	Cal	0.4546	2093	0.4925	11941
BPA_WS-190724-08	Cal	0.7576	3746	0.7777	11991
BPA_WS-190724-09	Cal	1.5152	7521	1.4001	12293
BPA_WS-190724-10	Cal	3.0303	15818	2.7815	12393
BPA_WS-190724-11	Cal	7.5758	42330	7.2629	12333
BPA_WS-190724-12	Cal	15.152	91412	15.54	12330
blank	Sample		0	0	14756
8657388	Sample		369	0.1774	15631
8657388 rec	Sample		10282	1.6394	14143
8657389	Sample		1017	0.2671	15226
8657389 rec	Sample		9799	1.5419	14409
BPA_WS-190724-09	Cal	1.5152	8772	1.4992	13301
8657390	Sample		4702	0.8173	14184
8657390 rec	Sample		13881	2.1676	14149
8657391	Sample		543	0.2008	15575
8657391 rec	Sample		9270	1.427	14837
BPA_WS-190724-07	Cal	0.4546	2565	0.4975	14441
BPA_WS-190724-08	Cal	0.7576	4485	0.7903	14082
BPA_WS-190724-09	Cal	1.5152	9016	1.4736	13932
BPA_WS-190724-10	Cal	3.0303	17795	2.8905	13391
BPA_WS-190724-11	Cal	7.5758	48006	7.3782	13764
BPA_WS-190724-12	Cal	15.152	101399	15.702	13534

Batch: **BPA_I-MA-190724-QQQ5-LK-1**

Calibration curve



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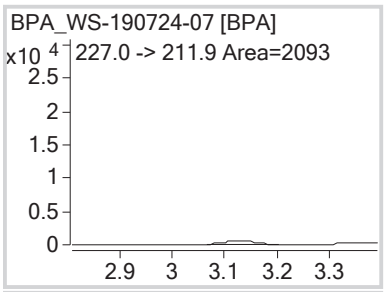

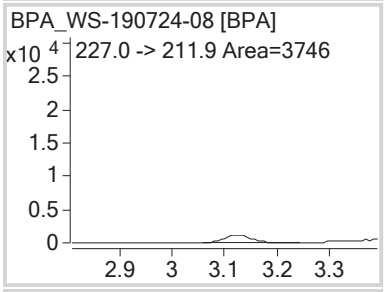

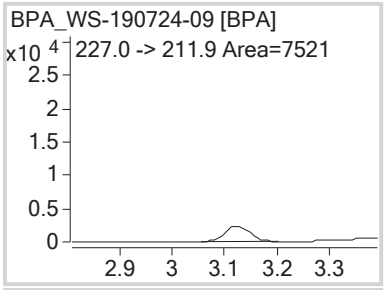

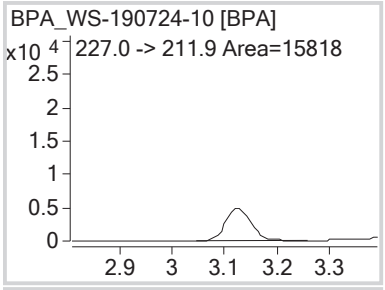

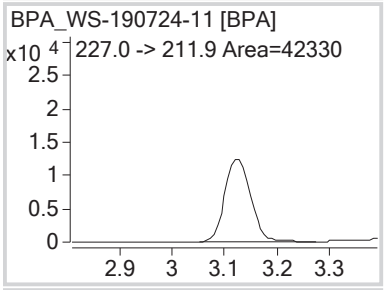

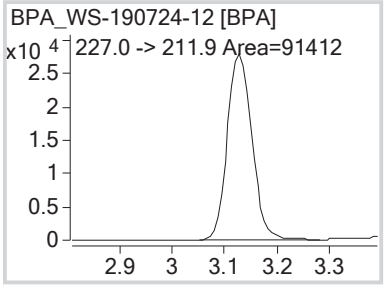

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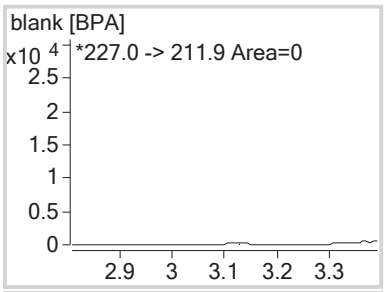

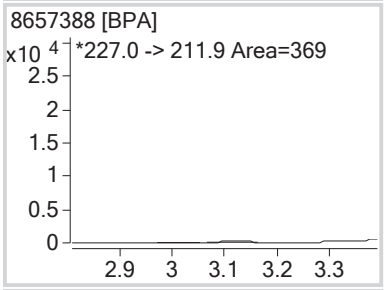

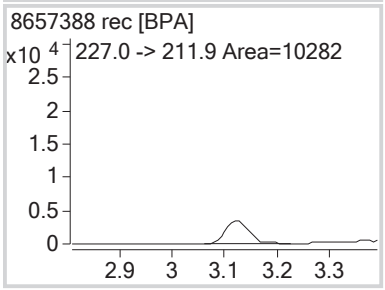

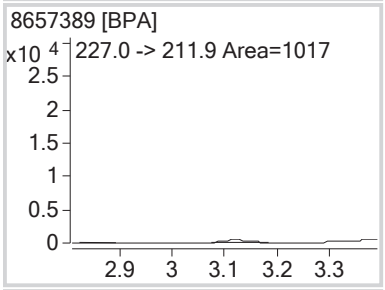

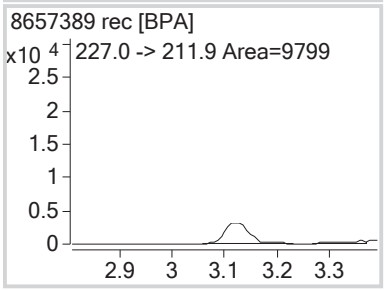

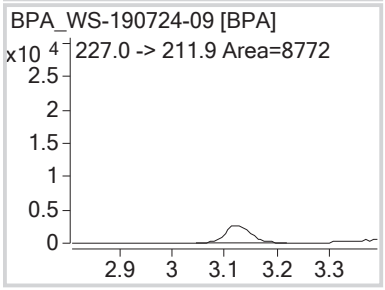

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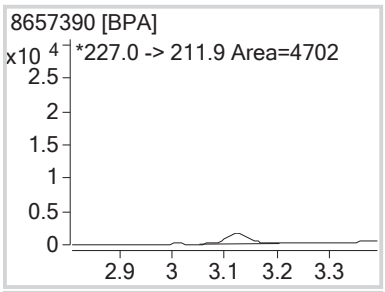

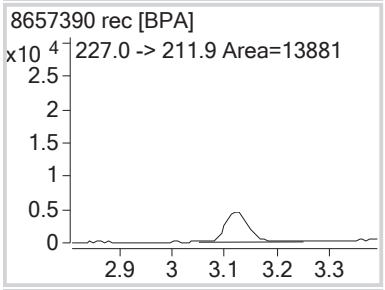

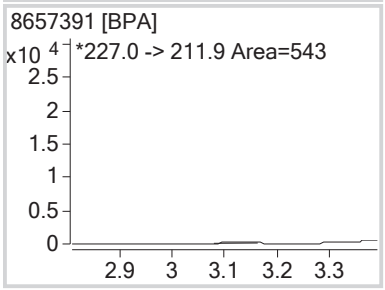

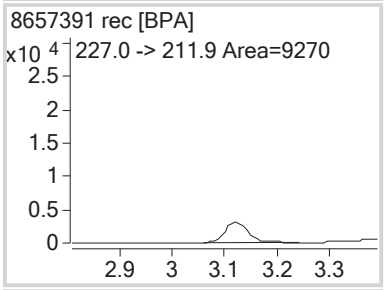

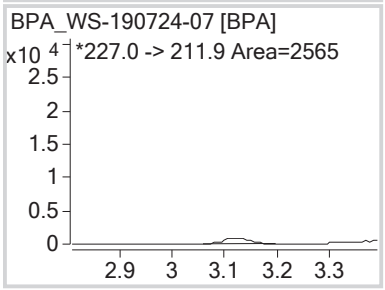

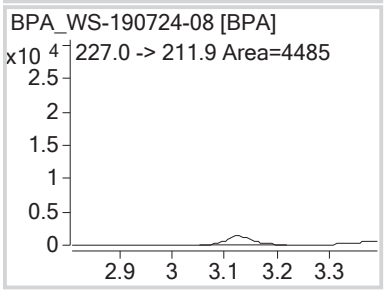

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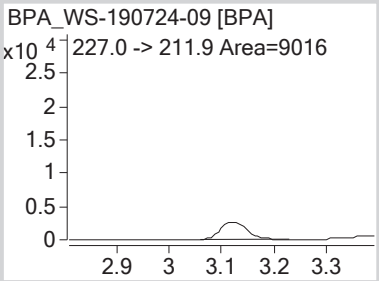
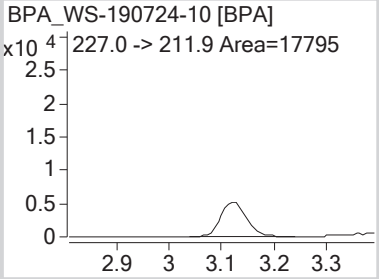
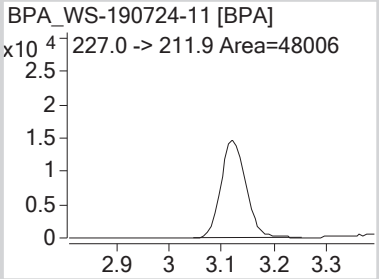






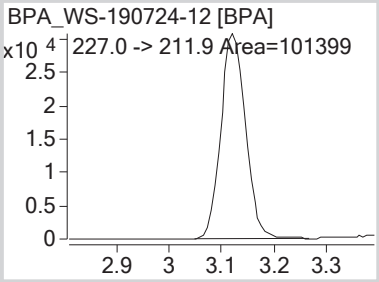


EXHIBIT C

Determination of BPA in Infant Formula, Commercial Packaged Non-Alcoholic Beverages, Pet Food and Food Packaging Migration Study Simulants by LC-MS/MS

AREA OF APPLICABILITY

This method applies to all facilities and business operations associated with Covance Food Solutions.

SCOPE

This method is applicable to the determination of bisphenol A (BPA) in infant formula, commercially packaged non-alcoholic beverages, pet food, and product simulants used in food packaging migration studies.

PRINCIPLE

BPA is extracted from a sample using 10 mL of 1% acetic acid in acetonitrile after an addition of stable-isotope labeled BPA internal standard (BPA-d16). Water (10 mL) is added to dry samples prior to the extraction step. Sodium chloride is used to salt out BPA into the acetonitrile phase. After centrifugation, a freeze-out step is used to remove co-extracted lipids. An aliquot of the supernatant upper layer is then analyzed using high pressure liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) in electrospray negative ionization mode. Food packaging migration study simulants are analyzed directly by LC-MS/MS after addition of the stable-isotope labeled internal standard.

PRECISION AND ACCURACY

The data that support the precision (measurement uncertainty) and accuracy for this assay are on file electronically and/or in an on-site central file.

LIMIT OF QUANTITATION (LOQ)

Limit of Quantitation (LOQ) for this assay are dependent on the matrix type and the sample preparation procedure. The following table lists the typical reporting limit (RL) for various matrices.

Matrix	Sample preparation	LOQ
Food Simulant in Packaging Migration Studies	Direct analysis	0.60 ng/g
Powdered Infant Formula	Sample extraction	2.0 ng/g
Liquids, Beverages, Ready-to-feed Infant Formula	Sample extraction	0.30 µg/L or 0.30 ng/g
Pet Food	Sample extraction	5.0 ng/g

REFERENCE

"Determination of Bisphenol A (BPA) in Commercially Packaged Ready to Consume Carbonated and Non-Carbonated Water and Non-Alcoholic Beverages", Covance developed method, accepted as AOAC First Action Official Method 2017.15

Supporting Reference

Shi, Z., Fu, H., Xu, D. Huai, Q., Zhang, H., "Salting-Out Assisted Liquid/Liquid Extraction Coupled with Low-Temperature Purification for Analysis of Endocrine-Disrupting Chemicals in Milk and Infant Formula by Ultra High Performance Liquid Chromatography-Tandem Mass Spectrometry," *Food Analytical Methods, 10* (5): 1523-1534 (2017)

DOCUMENT REVISION HISTORY

Version	Description of Revisions	Reason for Change	Effective Date
1	• Method Release	N/A	01 Sep 17
2	• Addition of method reference • Addition of pet food into the scope of the method, including LOQ information and modification of the calibration working standards and internal standard concentration • Modification of the Interferences section • Modified recommendations for the preparation of a series of calibration standards	• The method was approved AOAC First Action Official Method in Dec 2017 • Pet food was validated and added to the scope of the method • Clarification • Inclusion of a calibration level corresponding to the LOQ in beverages	Current

REVISION CATEGORY

Category 1	This is a new/revised method. All personnel required to follow content must read this version and complete training.	
Category 2	This is a new/revised method. All personnel required to follow content must read this version.	x
Category 3	No significant change to method content – no requirement to read or train.	

METHOD APPROVAL

Management Approval*: _____ Date: _____

Brent A. Rozema
Associate Director, Specialty Testing
Covance Food Solutions

* Document copies distributed electronically will not show signatures. These are retained on the wet-signed master document.

SAFETY PRECAUTIONS

Observe all standard laboratory safety procedures as outlined in the Environmental, Health, and Safety section of the Covance Policies and Procedures Manual.

INTERFERENCES

BPA is a widely-used monomer for producing polymer materials. It is common to have minor BPA contamination from plastic containers and plastic labware. Additionally, as an environmental contaminant, BPA might be present in water, acetonitrile, and sodium chloride used for the preparation of samples and mobile phase, and could contribute to the detected BPA in the method blank. Every effort should be made to minimize the BPA contamination during the sample preparation steps. If an interference occurs that would impact accurate BPA quantitation (such as in coffee samples), an alternative MS/MS transition should be used for quantitation (*m/z* 227.0 > 133.0 instead of *m/z* 227.0 > 211.9 for BPA or *m/z* 241.1 > 142.0 instead of *m/z* 241.1 > 233.1 for BPA-d16 internal standard).

DATA ACCEPTABILITY

- Each sample batch should contain the following control samples: (1) one method blank (water), and (2) fortified recovery QC samples equivalent to 10% of the samples in a run, unless otherwise specified in the protocol, or sample analysis outline.
- Recovery levels to be spiked are performed on the selected samples at a level that will produce sufficient instrument response above the level endogenous to the sample. Spike recovery (calculated as marginal recovery after subtraction of the endogenous BPA concentration) should be within acceptable levels as defined by Guidelines for Dietary Supplements and Botanicals, Appendix K, p. 8 or the AOAC SMPR; see Appendix A for details, depending on the matrix.
- Ion ratios (calculated as qualifier MS/MS transition *vs.* quantitation transition ratio) in the samples should be within 30% (relative) of the average of ion ratios in the calibration standards for at least one qualifier MS/MS transition.
- The method blank should have ≤ 50% reporting limit level of BPA (corrected for the nominal sample mass of a sample). The BPA level detected in the method blank is subtracted from the level calculated to be present in the samples. Method blank above this BPA background level disqualified the run and the source of contamination should be investigated.
- The internal standard response in the samples should be within 50 – 150% (relative) of the IS response of the method blank. The result out of this range suggests possible stronger matrix suppression/enhancement or poor BPA recovery. In these instances, standard addition can be used for BPA quantification.

SYSTEM SUITABILITY

- The calibration curves must have a coefficient of determination (r^2) of ≥0.995 to be acceptable.
- Calibration curve residuals (relative error) must be ≤15% for the lowest calibration level, and ≤10% for the other calibration levels.
- Analyte retention time in standards must have an RSD of ≤5%.

APPARATUS

- Analytical balance*, sensitive to at least 0.001 g
- Pipetters*, various sizes with tips
- Beakers, various sizes
- Volumetric flasks*, glass, Class A, TC, 10 and 25 mL
- 50 mL polypropylene centrifuge tubes
- 15 mL polypropylene centrifuge tubes
- 5 ¾" glass Pasteur pipets
- Autosampler vials
- Vortex mixer
- Horizontal shaker
- Centrifuge capable of 3000 g and maintaining < 5 °C. (e.g. 4000 rpm for a centrifuge with a 14 cm rotor)
- HPLC
  - Autoinjector, refrigerated
  - Pump, at least a binary pump with pressure operating range of up to at least 800 bar
  - LC Column, Agilent ZORBAX RRHD Eclipse Plus C18, 100 × 2.1 mm, 1.8 µm – Part Number 959758-902
  - LC column guard: Agilent ZORBAX Eclipse Plus C18, 2.1 × 5 mm, 1.8 µm – Part Number 821725-901
  - Column oven capable of maintaining at 40 ± 2 °C
- Mass spectrometer, Agilent 6495 triple quadrupole or equivalent

Note: Equivalent equipment may be substituted and will be documented in the raw data.

* Potential sources of measurement uncertainty (including the operator).

REAGENTS

- Ultrapure water (UPW)
- Acetonitrile, LC-MS grade
- Acetic acid, ACS grade

- Ammonium fluoride in water (40%) or ammonium fluoride, ACS grade
- Sodium chloride, ACS grade

Note: Equivalent reagents may be substituted and will be documented in the raw data.

REFERENCE MATERIALS (STANDARDS)

- Bisphenol A*, ≥99%, Sigma-Aldrich (catalog # 239658)
- Bisphenol A-d16, ≥98%, Cambridge Isotope Laboratories Inc. (catalog # DLM-1839-1)

Note: Equivalent reference materials may be substituted and will be documented in the raw data. Internal standards may also be provided by suppliers in solution.

* Potential sources of measurement uncertainty.

PROCEDURE

Note: Preparations may be scaled as needed.

Reagent Solution Preparation

Mobile Phase A (1 mM ammonium fluoride in water): Add 500 mL UPW to a 500 mL glass mobile phase reservoir. Add 42 µL of the 40% ammonium fluoride in water solution. Mix thoroughly. This solution is stable for 1 week when stored at room temperature.

Alternative: Add 19 mg ammonium fluoride instead of 40% ammonium fluoride in water.

Extraction solution (1% acetic acid in acetonitrile): Add 5 mL of acetic acid to a 500 mL glass mobile phase reservoir. Add acetonitrile to 500 mL. Mix thoroughly. This solution is stable for 6 months when stored at room temperature.

Standard Solutions Preparation

BPA Stock Solution 1 (200 µg/mL, BPA-SS1). Weigh 0.0050 g of bisphenol A into a 25 mL volumetric flask. Dilute to volume with acetonitrile. Mix well. Calculate the concentration in µg/mL correcting for reference standard purity. The solution is stable for 3 months when stored refrigerated.

BPA Stock Solution 2 (400 ng/mL, BPA-SS2). Transfer 20 µL of BPA-SS1 to a 10 mL volumetric flask. Dilute to volume with acetonitrile and mix well. The solution is stable for 3 months when stored refrigerated.

BPA Intermediate Standard Solutions (BPA-IWS). Sequentially prepare the BPA-IWS solutions in 1.8 mL autosampler vials and bringing to the total volume of 1 mL with acetonitrile. Mix well. The following scheme is recommended for preparation of a series (2, 20, 200 ng/mL) of BPA-IWS. Different calibration stock solution concentrations and volumes can be used as needed. The solution is stable for 7 days when stored refrigerated.

Intermediate Standard	BPA-SS2 (mL)	ACN (mL)	BPA concentration (ng/mL)
BPA-IWS1	0.500	0.500	200
BPA-IWS2	0.050	0.950	20
BPA-IWS3	0.005	0.995	2

Bisphenol A Internal Standard Stock Solution 1 (200 µg/mL, BPA-ISS1). Weigh 0.0050 g of bisphenol A-d16 into a 25 mL volumetric flask. Dilute to volume with acetonitrile and mix well. The solution is stable for 3 months when stored refrigerated.

Internal Standard Stock Solution 2 (2,000 ng/mL, BPA-ISS2). Transfer 100 µL of the BPA-ISS1 to a 10 mL volumetric flask. Dilute to volume with acetonitrile and mix well. The solution is stable for 3 months when stored refrigerated.

Internal Standard Intermediate Standard Solution 3 (200 ng/mL, BPA-ISS3). Prepare 1 mL BPA-ISS3 solution by adding 100 µL of BPA-ISS2 into a 1.8 mL autosampler vial bringing to the total volume of 1 mL with 0.9 mL acetonitrile. Mix well. The solution is stable for 7 days when stored refrigerated.

Internal Standard Intermediate Standard Solution 4 (20 ng/mL, BPA-ISS4). Prepare 1 mL BPA-ISS4 solution by adding 100 µL of BPA-ISS3 into a 1.8 mL autosampler vial bringing to the total volume of 1 mL with 0.9 mL acetonitrile. Mix well. The solution is stable for 7 days when stored refrigerated.

Solvent based calibration standard working solutions (BPA-WS). Prepare the BPA-WS solutions by combining appropriate volumes of the BPA-IWS solutions and BPA-ISS4 solution into a 1.8 ml autosampler vial and bringing to the total volume of 1.2 or 1 mL with acetonitrile. Mix each solution well. Recommendations for the preparation of a series of BPA-WS for pet food and other sample type analysis are listed below. Each standard used for the pet food analysis contains approximately 3 ng/mL of the labelled internal standard. Each standard used for the analysis of all other sample types contains approximately 1 ng/mL of the labelled internal standard. Different calibration stock solution concentrations and volumes can be used as needed. The solutions are stable for 7 days when stored refrigerated.

For pet food analysis:

Working Standard	BPA-ISS4 (mL)	BPA_IWS Name	BPA_IWS Conc. (ng/mL)	BPA_IWS Volume (mL)	ACN (mL)	Final Volume (mL)	BPA Conc. (ng/mL)	IS Conc. (ng/mL)
BPA-WS1	0.180	BPA-IWS2	20	0.027	0.993	1.2	0.45	3.0
BPA-WS2	0.180	BPA-IWS2	20	0.045	0.975	1.2	0.75	3.0
BPA-WS3	0.180	BPA-IWS2	20	0.090	0.930	1.2	1.5	3.0
BPA-WS4	0.180	BPA-IWS2	20	0.180	0.840	1.2	3.0	3.0
BPA-WS5	0.180	BPA-IWS1	200	0.045	0.975	1.2	7.5	3.0
BPA-WS6	0.180	BPA-IWS1	200	0.090	0.930	1.2	15	3.0

For analysis of all other sample types:

Working Standard	BPA-ISS4 (mL)	BPA_IWS Name	BPA_IWS Conc. (ng/mL)	BPA_IWS Volume (mL)	ACN (mL)	Final Volume (mL)	BPA Conc. (ng/mL)	IS Conc. (ng/mL)
BPA-WS1	0.050	BPA-IWS3	2	0.100	0.850	1.0	0.2	1.0
BPA-WS2	0.050	BPA-IWS3	2	0.150	0.800	1.0	0.3	1.0
BPA-WS3	0.050	BPA-IWS2	20	0.050	0.900	1.0	1.0	1.0
BPA-WS4	0.050	BPA-IWS2	20	0.100	0.850	1.0	2.0	1.0
BPA-WS5	0.050	BPA-IWS1	200	0.050	0.900	1.0	10	1.0
BPA-WS6	0.050	BPA-IWS1	200	0.100	0.850	1.0	20	1.0

Sample Preparation

Note: Always use an internal standard solution in the samples that is from the same stock preparation as that used for the standards.

Food simulants (from food packaging migration studies)

1 Weigh 1.00 ± 0.02 g of sample into an injection vial (1.00 g UPW is prepared with each sample set as the method blank).

2 For the fortified recovery sample, add an appropriate volume of the BPA spiking solution (BPA-IWS2).

3 Add 0.05 mL of the internal standard stock solution (BPA-ISS4, 20 ng/mL) to the injection vial. Mix well and proceed to LC-MS/MS analysis.

All other sample types

1 Take sample aliquot

1.1 Powders and pet foods: Weigh 1.50 ± 0.05 g of sample into a tared 50 mL polypropylene centrifuge tube.

1.2 Liquid products: Weigh 10.0 ± 0.2 g of sample into a tared 50 mL polypropylene centrifuge tube.

1.3 Method blank: Weigh 10.0 ± 0.2 g of UPW into a tared 50 mL polypropylene centrifuge tube. This method blank is prepared along with each sample set.

Note: The sample and method blank may also be taken and reported based on volume when requested by client.

2 For the fortified recovery sample, add an appropriate volume of the BPA spiking solution (BPA-IWS1).

3 Add internal standard

3.1 Add 0.15 mL of the internal standard intermediate standard solution (BPA-ISS3, 200 ng/mL) to pet food samples and the concurrently analyzed method blank. Vortex the sample for at least 15 seconds.

3.2 Add 0.05 mL of the internal standard intermediate standard solution (BPA-ISS3, 200 ng/mL) to the all other sample types and the corresponding method blank. Vortex the sample for at least 15 seconds.

Note: It is required that the internal standard used for preparation of both samples and standards originate from the same internal standard stock solution.

4 Add 10 mL UPW to dry samples. Vortex the sample for at least 15 seconds.

5 Add 10 mL of extraction solution to ALL samples. Cap the tube tightly and shake for ~10 minutes on a horizontal shaker set at approximately 200 rpm.

6 Add 2.2 ± 0.2 g of sodium chloride into a 15 mL polypropylene centrifuge tube.

7 Transfer 12 mL of the sample extract into the 15 mL polypropylene centrifuge tube containing sodium chloride. Invert the tube for at least 5 times to disperse the salt into the solution.

8 Place the tube on a horizontal shaker set at approximately 200 rpm for ~15 minutes.

9 Centrifuge the tube at 3000 RCF for 20 minutes at < 4 °C.

10 Keep the centrifuge tubes at -20 ± 5 °C for at least 30 minutes for fat precipitation.

11 Transfer a portion of the upper layer supernatant into an injection vial.

Notes: The sample extract is stable for 7 days when stored refrigerated and quantitated against the standards prepared at the same time.

CHROMATOGRAPHIC CONDITIONS

HPLC Parameters

Analytical Column	Agilent ZORBAX RRHD Eclipse Plus C18, 100 × 2.1 mm, 1.8 µm
Guard Column	Agilent Eclipse Plus C18, 5 × 2.1 mm, 1.8 µm
Column Oven	40 ± 2 °C
Injection Volume	3 µL
Autosampler	5 ± 2 °C
Mobile Phase A	1 mM ammonium fluoride in water
Mobile Phase B	acetonitrile

Gradient Settings

Time (minutes)	Flow (mL/min)	%A	%B
0	0.5	70	30
0.5	0.5	70	30
3.5	0.5	40	60
3.6	0.5	5	95
5.6	0.5	5	95
5.7	0.5	70	30
8.0	0.5	70	30

Notes:
At 2.3 minutes, the divert valve changes to MS position
At 3.3 minutes, the divert valve changes to waste position

Agilent 6495 LC-MS/MS Parameters

MS Acquisition	MRM
Ion source type	AJS ESI-
Drying gas temperature, °C	250
Drying gas flow, L/min	15
Nebulizer, psi	60
Sheath gas heater, °C	330
Sheath gas flow, L/min	12
Capillary, V	3000
Nozzle voltage, V	1000
Negative high pressure RF	90 V
Negative low pressure RF	60 V
Precursor ion and product ion resolution	Unit

MS/MS Transitions

Compound	Precursor Mass (m/z)	Product Mass (m/z)	Collision Energy (V)	Cell Accelerator (V)	Retention Time (min)
Bisphenol-A	227.0	211.9	18	3	2.7
	227.0	133.0	28	3	
	227.0	93.1	53	3	
Bisphenol A-d16	241.1	223.1	19	3	2.7
	241.1	142.0	30	3	

The following quantification transitions are typically used:
m/z 227.0 > 211.9 for BPA and *m/z* 241.1>223.1 for the BPA-d16 internal standard. Alternative MS/MS transitions *m/z* 227.0>133.0 and *m/z* 241.1>142.0 can be used for samples with interferences affecting accurate integration of the BPA or BPA-d16 peaks, such as in coffee or pet food samples, respectively.

Note: These guidelines may be modified to obtain desired chromatography. Any modifications will be documented in the raw data. Not all of the above listed transitions need to be used. Some matrices have interferences present on some of the transitions.

Injection Sequence

- A standard is injected, at a minimum, between every ten samples, duplicates, or method blanks.
- Each analytical sequence is bracketed by at least two standards at the beginning of the sequence and at least one standard at the end of the sequence.
- A total of at least four different standard concentrations are required to establish a calibration curve.

CALCULATIONS

1 Integrate BPA in each standard and sample injection.

2 Calibration is performed using 1/x weighted linear regression where the abscissa (x-axis) is the external working standard concentration (ng/mL) and the ordinate (y-axis) is a response factor defined as:

$$Response\ factor = \frac{A}{\mathrm{A_{Istd}}}$$

Where:
A = analyte peak area
A_{Istd} = ISTD peak area

3 Calculate the concentration of BPA in the sample using the following calculation:

$$BPA = \frac{C_{\text{sample extract}} - C_{\text{method blank}}}{S} \times \frac{C_{\mathrm{IS}} \times V_{\mathrm{IS}}}{C_{\mathrm{ISTD}}}$$

Where:
BPA (ng/g or µg/L) = Reported concentration of BPA in the sample
$C_{sample\ extract}$ (ng/mL) = BPA concentration in the sample extract as calculated from the calibration curve
$C_{method\ blank}$ (ng/mL) = BPA concentration in the method blank extract as calculated from the calibration curve
C_{ISTD} (ng/mL) = Concentration of the internal standard in calibration working standards BPA-WS
C_{IS} (ng/mL) = Concentration of the internal standard solution added to the sample and method blank
V_{IS} (mL) = Volume of the internal standard solution added to the sample and method blank
S (g or mL) = Sample and method blank weight or volume

4 Standard Addition:

For standard addition, the sample is prepared with four (or more) test portions. One portion is analyzed as such (native), and known amounts of the BPA standard are added to the other test portions. The amount of the analyte standard added should be between 100% and 500% of the estimated or solvent based amount of the analyte in the native sample. The analyte concentration in the sample is derived from the x-intercept of a linear regression of the analyte peak areas in the native and standard addition samples and the added concentrations. This procedure is designed to determine the content of an analyte in a sample, inherently

taking into account the recovery of the analytical procedure and also compensating for any matrix effect.

For routine analysis, it is recommended to use at least a 3-level standard addition (at 100%, 200%, and 400% of the estimated or solvent based concentration and generate a 4-point linear regression curve which includes the native extract (coefficient of determination $r^2 \geq 0.99$) for standard addition calculation.

Calculate a linear regression curve ($y = ax + b$) with the added concentration in ng/g as the abscissa (x-axis) and the response factor as the ordinate (y-axis). The native extract is included in the regression using a concentration of zero (0).

The analyte concentration in the sample is calculated:

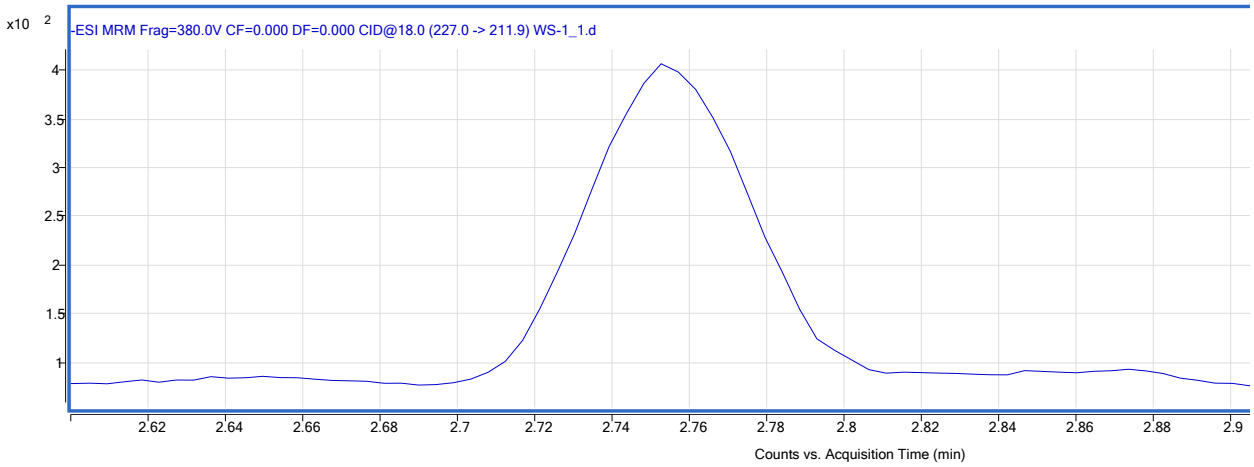
$$BPA = \frac{|-b|}{a}$$

Where:

BPA = Reported concentration of analyte in the sample in ng/g
a = Slope of the linear regression curve
b = Intercept of the linear regression curve

EXAMPLE CHROMATOGRAPHY

Extracted ion chromatogram (*m/z* 227.0 > 211.9) of a low BPA standard at approximately 0.1 ng/mL



APPENDIX A

SPIKE RECOVERY GUIDELINES

The requirements of QC spikes recovery for commercially packaged beverages follow the AOAC SMPR. The QC spike recovery of other matrices follows AOAC Official Methods of Analysis.

Taken from *Official Methods of Analysis of AOAC International*, "Guidelines for Dietary Supplements and Botanicals", Appendix K, p. 8.

Acceptable recovery is a function of the concentration and the purpose of the analysis. Some acceptable recovery requirements for individual assays are as follows:

Concentration	Recovery limits, %
100%	98-101
10%	95-102
1%	92-105
0.1%	90-108
0.01%	85-110
10 µg/g (ppm)	80-115
1 µg/g (ppm)	75-120
10 µg/kg (ppb)	70-125

Taken from "AOAC SMPR draft (version 7; July 11, 2017) – Determination of free Bisphenol A (BPA) in commercially packaged ready to consume carbonated and non-carbonated water and non-alcoholic beverages".

Limit of Detection (LOD)		≤ 0.1 µg/liter	
Limit of Quantitation (LOQ)		≤ 0.5 µg/liter	
Analytical range*	< 2 µg/liter	2 – 5 µg/liter	5 – 20 µg/liter
Accuracy	60% – 140%	80% – 120%	80% – 120%
% RSD_r	≤ 20%	≤ 10%	≤ 5%
% RSD_R	≤ 40%	≤ 20%	≤ 10%
Units are expressed as µg/liter as weight/volume. * Concentration in the ready to drink product			

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Method Mnemonic:	BPA_S
Method Sub-Mnemonic:	NA

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1) AREA OF APPLICABILITY

This method applies to all facilities and business operations associated with Eurofins Food Integrity & Innovation.

2) SCOPE

This method is applicable to the determination of bisphenol A (BPA) in infant formula, commercially packaged non-alcoholic beverages, pet food, botanicals, food matrices, and product simulants used in food packaging migration studies.

3) PRINCIPLE

BPA is extracted from a sample using 10 mL of 1% acetic acid in acetonitrile after an addition of stable-isotope labeled BPA internal standard (BPA-d_{16}). Water (10 mL) is added to dry samples prior to the extraction step. Sodium chloride is used to salt out BPA into the acetonitrile phase. After centrifugation, a freeze-out step is used to remove co-extracted lipids. An aliquot of the supernatant upper layer is then analyzed using high pressure liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) in electrospray negative ionization mode. Food packaging migration study simulants are analyzed directly by LC-MS/MS after addition of the stable-isotope labeled internal standard.

4) SAFETY PRECAUTIONS

Observe all standard laboratory safety procedures as outlined in local Eurofins Environmental Health and Safety (EHS) policies and procedures.

5) LIMIT OF QUANTITATION (LOQ)

Limit of Quantitation (LOQ) for this assay are dependent on the matrix type and the sample preparation procedure. The following table lists the typical reporting limit (RL) for various matrices.

Matrix	Sample Preparation	LOQ
Food Simulant in Packaging Migration Studies	Direct analysis	0.60 ng/g
Powdered Infant Formula	Sample extraction	2.0 ng/g
Liquids, Beverages, Ready-to-feed Infant Formula, Food Matrices	Sample extraction	0.30 µg/L or 0.30 ng/g
Pet Food, Botanicals	Sample extraction	5.0 ng/g

6) INTERFERENCES

BPA_S:Determination of BPA by LC-MS_MS

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BPA is a widely-used monomer for producing polymer materials. It is common to have minor BPA contamination from plastic containers and plastic labware. Additionally, as an environmental contaminant, BPA might be present in water, acetonitrile, and sodium chloride used for the preparation of samples and mobile phase, and could contribute to the detected BPA in the method blank. BPA may also be present in dust; therefore, it is imperative to maintain a clean work space or prepare samples in a clean room (or hood) whenever possible. Every effort should be made to minimize contamination with BPA during the sample preparation steps.

7) DATA ACCEPTABILITY & SYSTEM SUITABILITY

Quality Control Acceptance Criteria:

Type	Requirement
Minimum Number of QC Samples per run	Each sample batch should contain the following control samples: one method blank (UPW), and a number of fortified recovery QC samples equivalent to at least 10% of the samples in a run, unless otherwise specified in the protocol, or sample analysis outline.
Spike Recoveries	Spiking should be performed at concentrations that produce sufficient instrument response above the level endogenous to the sample. Marginal spike recovery for samples other than commercially packaged beverages should be within 70-125%. Refer to Section 17 (Appendix B) for acceptable marginal recovery range in commercially packaged beverages.

System Suitability / Data Acceptance Criteria:

Type	Requirement
Injection Order	A minimum of three equilibration injections consisting of two working standards and one UPW blank should precede the first set of standards used for calibration. A standard is injected, at a minimum, between every ten samples, duplicates, or method blanks. The method blank should be injected after the first set of standards.
Bracketing Order	Each analytical sequence is bracketed by at least two standards at the beginning of the sequence and at least one standard at the end of the sequence.
Minimum No of Calibration Standards	A total of at least four different standard concentrations covering analyte reportable levels are required to establish a calibration curve. Not more than 25% of the standard

BPA_S:Determination of BPA by LC-MS_MS

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	injections not meeting the requirements below can be eliminated from the calibration.
Coefficient of Determination (r2)	≥ 0.995
Calibration Standards (Level 1)	±15% of theoretical value
Calibration Standards (Levels 2-5)	±10% of theoretical value
Blank (Method)	The method blank should have concentration ≤ 50% reporting limit level of BPA (corrected for the nominal sample mass of a sample). Method blank above this BPA background level disqualifies the run and the source of contamination should be investigated. The BPA level detected on instrument in the method blank is subtracted from the on instrument level calculated to be present in the samples to account for any contamination arising from reagents, consumables, or the LCMS system.
Qualifier Ion Ratios	At least two multiple reaction monitoring (MRM) transitions are monitored for BPA. For a result to be reported, the ratio(s) of MRMs in the samples should be within 30% (relative) of the average ratio established in the calibration standards in the same analysis batch.
Internal Standard Recovery	The internal standard (IS) response in the samples should be within 50 – 150% (relative) of the IS response in the method blank. IS response within 20-150% of the IS response in method blank is acceptable for propolis samples. The result out of this range suggests possible stronger matrix suppression/enhancement or poor BPA recovery. In these instances, standard addition can be used for BPA quantification.
%RSD of Analyte Retention Time in Calibration Standards	≤ 5%

8) APPARATUS

Where a particular brand or source of a material, instrument or piece of equipment, or the name and address of a manufacturer or distributor, is mentioned, this identification is furnished solely for informational purposes as a matter of convenience. Items capable of equal or better performance may be used if these characteristics have been verified.

Where low-actinic or light-resistant containers are specified, clear containers that have been rendered opaque by application of a suitable coating or wrapping may be used.

- Analytical balance*, sensitive to at least 0.001 g

BPA_S:Determination of BPA by LC-MS_MS

- Pipetters*, various sizes with tips
- Beakers, various sizes
- Volumetric flasks*, glass, Class A, TC, 10 and 25 mL
- Volumetric pipets*, Class A, TD, 10 mL
- 50 mL polypropylene centrifuge tubes
- 15 mL polypropylene centrifuge tubes
- 5 ¾" glass Pasteur pipets
- Autosampler vials
- Vortex mixer
- Horizontal shaker
- Centrifuge capable of 3000 g and maintaining < 5 °C. (e.g. 4000 rpm for a centrifuge with a 14 cm rotor)
- HPLC
  - Autoinjector, refrigerated
  - Pump, at least a binary pump with pressure operating range of up to at least 800 bar
  - LC analytical column, Agilent ZORBAX RRHD Eclipse Plus C18, 100 × 2.1 mm, 1.8 µm (Part number 959758-902)
  - LC guard column: Agilent ZORBAX Eclipse Plus C18, 2.1 × 5 mm, 1.8 µm (Part number 821725-901)
  - Column oven capable of maintaining 40 ± 2 °C
- Mass spectrometer, Agilent 6495 triple quadrupole or equivalent

* Potential sources of measurement uncertainty (including the operator).

9) REAGENTS & SOLUTIONS

9.1 Reagents

Where a particular brand or source of a material, or the name and address of a manufacturer or distributor, is mentioned, this identification is furnished solely for informational purposes as a matter of convenience. Material of equal or better purity or specification may be used.

- Ultrapure water (UPW)
- Acetonitrile, LC-MS grade
- Acetic acid, ACS grade
- Ammonium fluoride in water (40%) or ammonium fluoride, ACS grade
- Sodium chloride, ACS grade

Note: Equivalent reagents may be substituted and will be documented in the raw data.

9.2 Solutions

Mobile Phase A (1 mM ammonium fluoride in UPW)

- Add 500 mL UPW to a 500 mL glass mobile phase reservoir.
- Add 42 µL of the 40% ammonium fluoride in water solution.
- Mix thoroughly.
- This solution is stable for 1 week when stored at room temperature.
  - Alternative: Add 19 mg of ammonium fluoride instead of 40% ammonium fluoride in water.

BPA_S:Determination of BPA by LC-MS_MS

Extraction solution (1% acetic acid in acetonitrile)

- Add 5 mL of acetic acid to a 500 mL glass mobile phase reservoir.
- Add acetonitrile to 500 mL.
- Mix thoroughly.
- This solution is stable for 6 months when stored at room temperature.

10) REFERENCE MATERIALS & STANDARDS

10.1 Reference Materials

Where possible, reference materials are either purchased from ISO17034 accredited producers, or traceable to SI units of measurement/certified reference materials.

- Bisphenol A*, ≥99%, Sigma-Aldrich (catalogue number 239658)
- Bisphenol A-d_{16}, ≥98%, Cambridge Isotope Laboratories Inc. (catalogue number DLM-1839-1)
  - Internal standards may also be provided by suppliers in solution.

* Potential sources of measurement uncertainty.

Note: Equivalent reference materials may be substituted and will be documented in the raw data.

10.2 Standard Preparation

Preparations of standards may be scaled as needed. Proportionately larger or smaller quantities than the specified weights and volumes may be taken provided the measurement is made with at least equivalent accuracy.

BPA Stock Solution 1 (200 µg/mL, BPA-SS1)

- Weigh 0.0050 g of bisphenol A into a 25 mL volumetric flask.
- Dilute to volume with acetonitrile.
- Mix well.
- Calculate the concentration in µg/mL correcting for reference standard purity.
- The solution is stable for 3 months when stored refrigerated.

BPA Stock Solution 2 (400 ng/mL, BPA-SS2)

- Transfer 20 µL of BPA-SS1 to a 10 mL volumetric flask.
- Dilute to volume with acetonitrile and mix well.
- The solution is stable for 3 months when stored refrigerated.

BPA Intermediate Standard Solutions (BPA-IWS)

- Sequentially prepare the BPA-IWS solutions in 1.8 mL autosampler vials and bringing to the total volume of 1 mL with acetonitrile.
- Mix well.
- The following scheme is recommended for preparation of a series (2, 20, 200 ng/mL) of BPA-IWS.
- Intermediate standard solutions can be scaled as needed. Any differences should be documented in the raw data.
- The solution is stable for 7 days when stored refrigerated.

Intermediate			

BPA_S:Determination of BPA by LC-MS_MS

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Standard	BPA-SS2 (mL)	ACN (mL)	BPA concentration (ng/mL)
BPA-IWS1	0.500	0.500	200
BPA-IWS2	0.050	0.950	20
BPA-IWS3	0.005	0.995	2

Bisphenol A Internal Standard Stock Solution 1 (200 µg/mL, BPA-ISS1)

- Weigh 0.0050 g of bisphenol A-d_{16} into a 25 mL volumetric flask.
- Dilute to volume with acetonitrile and mix well.
- The solution is stable for 3 months when stored refrigerated.

Internal Standard Stock Solution 2 (2,000 ng/mL, BPA-ISS2)

- Transfer 100 µL of the BPA-ISS1 to a 10 mL volumetric flask.
- Dilute to volume with acetonitrile and mix well.
- The solution is stable for 3 months when stored refrigerated.

Internal Standard Intermediate Standard Solution 3 (200 ng/mL, BPA-ISS3)

- Prepare 1 mL BPA-ISS3 solution by adding 100 µL of BPA-ISS2 into a 1.8 mL autosampler vial bringing to the total volume of 1 mL with 0.9 mL acetonitrile.
- Mix well.
- The solution is stable for 7 days when stored refrigerated.

Internal Standard Intermediate Standard Solution 4 (20 ng/mL, BPA-ISS4)

- Prepare 1 mL BPA-ISS4 solution by adding 100 µL of BPA-ISS3 into a 1.8 mL autosampler vial bringing to the total volume of 1 mL with 0.9 mL acetonitrile.
- Mix well.
- The solution is stable for 7 days when stored refrigerated.

Solvent based calibration standard working solutions (BPA-WS)

- Prepare the BPA-WS solutions by combining appropriate volumes of the BPA-IWS solutions and BPA-ISS4 solution into a 1.8 ml autosampler vial and bringing to the total volume of 1.2 or 1 mL with acetonitrile.
- Mix each solution well.
- Recommendations for the preparation of a series of BPA-WS for analysis of pet food, as well as other sample types, are listed in the tables below
- Calibration standard working solutions can be scaled as needed, or calibration standard working solutions with a different final BPA concentration may be used providing the analytical range of the assay is not exceeded. Any differences should be documented in the raw data.
- The solutions are stable for 7 days when stored refrigerated.

Note: Each standard used for the pet food analysis contains approximately **3 ng/mL of IS**, while each standard used for the analysis of all other sample types contains approximately **1 ng/mL of IS**.

For pet food analysis:

Working Standard	BPA-ISS4 (mL)	BPA_IWS Name	BPA_IWS Concentration (ng/mL)	BPA_IWS Volume (mL)	ACN (mL)	Final Volume (mL)	Nominal BPA Concentration (ng/mL)	IS Concentra (ng/mL
BPA-WS1	0.180		20	0.027	0.993	1.2	0.45	3.0

BPA_S:Determination of BPA by LC-MS_MS

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		BPA-IWS2						
BPA-WS2	0.180	BPA-IWS2	20	0.045	0.975	1.2	0.75	3.0
BPA-WS3	0.180	BPA-IWS2	20	0.090	0.930	1.2	1.5	3.0
BPA-WS4	0.180	BPA-IWS2	20	0.180	0.840	1.2	3.0	3.0
BPA-WS5	0.180	BPA-IWS1	200	0.045	0.975	1.2	7.5	3.0
BPA-WS6	0.180	BPA-IWS1	200	0.090	0.930	1.2	15	3.0

For analysis of all other sample types:

Working Standard	BPA-ISS4 (mL)	BPA_IWS Name	BPA_IWS Concentration (ng/mL)	BPA_IWS Volume (mL)	ACN (mL)	Final Volume (mL)	Nominal BPA Concentration (ng/mL)	IS Concentra (ng/ml
BPA-WS1	0.050	BPA-IWS3	2	0.100	0.850	1.0	0.2	1.0
BPA-WS2	0.050	BPA-IWS3	2	0.150	0.800	1.0	0.3	1.0
BPA-WS3	0.050	BPA-IWS2	20	0.050	0.900	1.0	1.0	1.0
BPA-WS4	0.050	BPA-IWS2	20	0.100	0.850	1.0	2.0	1.0
BPA-WS5	0.050	BPA-IWS1	200	0.050	0.900	1.0	10	1.0
BPA-WS6	0.050	BPA-IWS1	200	0.100	0.850	1.0	20	1.0

11) PROCEDURE

- Always use an internal standard solution in the samples that is from the same stock preparation as that used for the standards.

11.1) Sample Preparation – Food Simulants (from food packaging migration studies)

11.1.1 Weigh 1.00 ± 0.02 g of sample into an injection vial.
- ± 0.02 g extraction solvent is prepared with each sample set as the method blank. If the extraction solvent is unknown or is a complicated mixture, then ACN is used as the method blank.
- For fortified recovery samples, a duplicate sample is weighed into an injection vial.

11.1.2 For the fortified recovery sample, add an appropriate volume of the BPA spiking solution (BPA-IWS2).

11.1.3 Add 0.05 mL of the internal standard stock solution (BPA-ISS4) to the injection vial. Mix well and proceed to LC-MS/MS analysis.

11.2) Sample Preparation – All Other Sample Types

11.2.1 Weigh appropriate amount of the sample into a tared 50 mL polypropylene centrifuge tube as indicated below:

BPA_S:Determination of BPA by LC-MS_MS

Sample Type	Sample Weight
Formula powders, botanicals, and pet foods	Weigh 1.50 ± 0.05 g of sample
Liquid products and food matrices	Weigh 10.0 ± 0.2 g of sample
Method blank	Weigh 10.0 ± 0.2 g of UPW

- The method blank is prepared along with each sample set.
- For fortified recovery samples, a duplicate amount of the sample is weighed into a tared 50 mL polypropylene centrifuge tube.
- A liquid sample and method blank may also be aliquoted and reported based on volume when requested by the client. To perform volume aliquot, measure 10 mL of sample using a Class A volumetric pipet into a tared 50 mL polypropylene centrifuge tube. This exact volume is recorded with the raw data. It is recommended to also measure the volume addition by weight in case a unit conversion is required at a later date.

11.2.2 For the fortified recovery sample, add an appropriate volume of the BPA spiking solution (BPA-SS2).

11.2.3 Add appropriate volume of internal standard as indicated below to samples, method blanks, and fortified recovery samples. Vortex for at least 15 seconds.

Sample Type	Volume of BPA-ISS3
Pet food samples	0.15 mL
All other sample types	0.05 mL

11.2.4 Add 10 mL UPW to dry samples. Vortex the samples for at least 15 seconds.

11.2.5 Add 10 mL of extraction solution to ALL samples. Cap the tubes tightly and shake for ~10 minutes on a horizontal shaker set at approximately 200 rpm.

11.2.6 Add 2.2 ± 0.2 g of sodium chloride into a 15 mL polypropylene centrifuge tube.

11.2.7 Transfer 12 mL of the sample extract into the 15 mL polypropylene centrifuge tube containing sodium chloride. Invert the tube at least 5 times to disperse the salt into the solution.

11.2.8 Place the tube on a horizontal shaker set at approximately 200 rpm for ~15 minutes.

11.2.9 Centrifuge the tube at 3000 RCF for 20 minutes at <4 °C.

11.2.10 Keep the centrifuge tubes at -20 ± 5 °C for at least 30 minutes for fat precipitation.

11.2.11 Transfer a portion of the upper layer supernatant into an injection vial.

- The sample extract is stable for 7 days when stored refrigerated and quantitated against the standards prepared at the same time.

12) INSTRUMENTATION CONDITIONS

BPA_S:Determination of BPA by LC-MS_MS

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HPLC Parameters:

Analytical Column:	Agilent ZORBAX RRHD Eclipse Plus C18, 100 × 2.1 mm, 1.8 µm
Guard Column:	Agilent Eclipse Plus C18, 5 × 2.1 mm, 1.8 µm
Column Oven:	40 ± 2 °C
Injection Volume:	3 µL
Autosampler:	5 ± 2 °C
Mobile Phase A:	1 mM ammonium fluoride in UPW
Mobile Phase B:	Acetonitrile

Gradient Settings:

Time (minutes)	Flow (mL/min)	%A	%B
0	0.5	70	30
0.5	0.5	70	30
3.5	0.5	40	60
3.6	0.5	5	95
5.6	0.5	5	95
5.7	0.5	70	30
8.0	0.5	70	30

- At 2.3 minutes, the divert valve changes to MS position
- At 3.3 minutes, the divert valve changes to waste position

Agilent 6495 LC-MS/MS Parameters:

MS Acquisition	MRM
Ion source type	AJS ESI
Polarity	Negative
Drying gas temperature	250 °C
Drying gas flow	15 L/min
Nebulizer	60 psi
Sheath gas heater	330 °C
Sheath gas flow	12 L/min
Capillary	3000 V
Nozzle voltage	1000 V
Negative high pressure RF	90 V
Negative low pressure RF	60 V
Precursor ion and product ion resolution	Unit

MS/MS Transitions:

Compound	Precursor Mass (*m/z*)	Product Mass (*m/z*)	Collision Energy (V)	Cell Accelerator (V)	Retention Time (min)
Bisphenol-A	227.0	211.9	18	3	2.7
	227.0	133.0	28	3	
	227.0	93.1	53	3	
Bisphenol A-d_{16}	241.1	223.1	19	3	2.7
	241.1	142.0	30	3	

The following quantification transitions are typically used:

BPA_S:Determination of BPA by LC-MS_MS

- *m/z* 227.0 > 211.9 for BPA and *m/z* 241.1>223.1 for the BPA-d_{16} internal standard.
- In samples that may have interferences (e.g. coffee, pet food, and propolis), alternative MS/MS transitions *m/z* 227.0>133.0 and *m/z* 241.1>142.0 can be used for BPA and BPA-d_{16}, respectively.

These guidelines may be modified to obtain desired chromatography and instrument response. Any modifications will be documented in the raw data.
Note: If circumstances warrant, additional cleaning steps may be added to the non-analytical portion of the chromatographic method (i.e. after elution of the peaks of interest or prior to injection). These may include addition of rinse steps, gradients, or ramped flow rates to remove matrix interferences. If used, these additional steps are not considered to be method deviations.

Injection Order

- A minimum of three equilibration injections consisting of two working standards and one UPW blank should precede the first set of standards used for calibration.
- A method blank should be injected after the first set of standards used for calibration.
- A standard is injected, at a minimum, between every ten samples, duplicates, or method blanks.
- Each analytical sequence is bracketed by at least two standards at the beginning of the sequence and at least one standard at the end of the sequence.

13) CALCULATIONS

13.1 Integrate BPA and BPA-d_{16} peaks in each standard and sample injection.

13.2 Generate a calibration curve using 1/x weighted linear regression with the external working standard concentration (ng/mL) as abscissa (x-axis) and the response factor defined below as ordinate (y-axis).

$$\text{Response factor} = \frac{A}{A_{IS}}$$

Where:

A	=	analyte peak area
A_{IS}	=	internal standard peak area

13.3 Calculate the concentration of BPA in the sample using the following calculation:

$$\text{BPA (ng/g or ng/mL)} = \frac{(C_{\text{sample extract}} - C_{\text{method blank}})}{S} \times \frac{C_1 \times V_1}{C_2}$$

Where:

$C_{\text{sample extract}}$	=	BPA concentration in the sample extract as calculated from the calibration curve (ng/mL)
$C_{\text{method blank}}$	=	BPA concentration in the method blank extract as calculated from the calibration curve (ng/mL)
C_1	=	

BPA_S:Determination of BPA by LC-MS_MS

		Concentration of the internal standard solution added to the sample and method blank (ng/mL)
C_2	=	Concentration of the internal standard in calibration working standards BPA-WS (ng/mL)
V_1	=	Volume of the internal standard solution added to the sample and method blank (mL)
S	=	Sample and method blank weight or volume (g or mL)

13.4 Standard Addition:

- For standard addition, the sample is prepared with four (or more) test portions.
- One portion is analyzed as such (native), and known amounts of the BPA standard are added to the other test portions.
- The amount of the analyte standard added should be between 100% and 500% of the estimated or solvent based amount of the analyte in the native sample.
- The analyte concentration in the sample is derived from the x-intercept of a linear regression of the analyte peak areas in the native and standard addition samples and the added concentrations.
- This procedure is designed to determine the content of an analyte in a sample, inherently taking into account the recovery of the analytical procedure and also compensating for any matrix effect.

13.5 For routine analysis, it is recommended to use at least a 3-level standard addition (at 100%, 200%, and 400% of the estimated or solvent based concentration and generate a 4-point linear regression curve which includes the native extract (coefficient of determination $r^2 \geq 0.99$) for standard addition calculation.

13.6 Calculate a linear regression curve (y = ax + b) with the added concentration in ng/g (or ng/mL) as the abscissa (x-axis) and the analyte peak area as the ordinate (y-axis). The native extract is included in the regression using a concentration of zero (0).

13.7 The analyte concentration in the sample is calculated:

$$\text{BPA (ng/g or ng/mL)} = \frac{|-b|}{a}$$

Where:

a	=	Slope of the linear regression curve
b	=	Intercept of the linear regression curve

14) PRECISION AND ACCURACY

Data supporting precision (measurement uncertainty) and accuracy for this assay is on file electronically and/or in an on-site central file.

15) REFERENCES

"Determination of Bisphenol A (BPA) in Commercially Packaged Ready to Consume Carbonated and Non-Carbonated Water and Non-Alcoholic Beverages", Eurofins Food Integrity & Innovation developed method, accepted as AOAC First Action Official Method 2017.15.

BPA_S:Determination of BPA by LC-MS_MS

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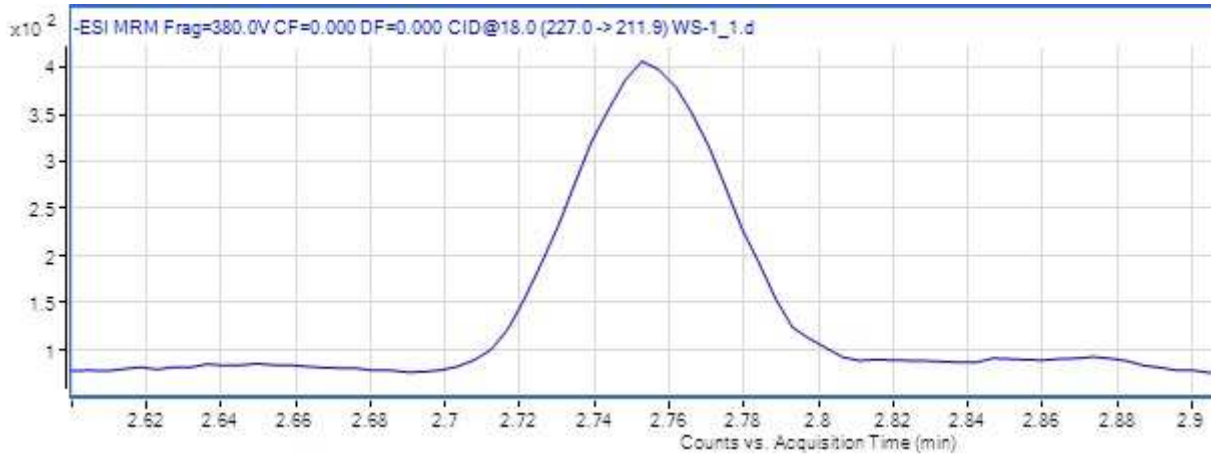
Supporting Reference

Shi, Z., Fu, H., Xu, D. Huai, Q., Zhang, H., "Salting-Out Assisted Liquid/Liquid Extraction Coupled with Low-Temperature Purification for Analysis of Endocrine-Disrupting Chemicals in Milk and Infant Formula by Ultra High Performance Liquid Chromatography-Tandem Mass Spectrometry," *Food Analytical Methods*, *10* (5): 1523-1534 (2017).

16) APPENDIX A - EXAMPLE CHROMATOGRAPHY

Extracted ion chromatogram (*m/z* 227.0 > 211.9) of a low BPA standard at approximately 0.1 ng/mL.



17) APPENDIX B - SPIKE RECOVERY GUIDELINES

Taken from "AOAC SMPR draft (version 7; July 11, 2017) – Determination of free Bisphenol A (BPA) in commercially packaged ready to consume carbonated and non-carbonated water and non-alcoholic beverages".

Limit of Detection (LOD)		≤ 0.1 µg/liter	
Limit of Quantitation (LOQ)		≤ 0.5 µg/liter	
Analytical range*	< 2 µg/liter	2 – 5 µg/liter	5 – 20 µg/liter
Accuracy	60% – 140%	80% – 120%	80% – 120%
% RSD_r	≤ 20%	≤ 10%	≤ 5%
% RSD_R	≤ 40%	≤ 20%	≤ 10%
Units are expressed as µg/liter as weight/volume. * Concentration in the ready to drink product			

End of document

BPA_S:Determination of BPA by LC-MS_MS

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Version	Approval	Revision information
1	18.JUL.2018	
2	06.MAR.2019	Updated to new format. Updated to inlcude method extension for analysis of black walnut wormwood extract, propolis, applesauce, MCT oil, and salmon representing botanical and food matrices. Updated title to be more generic as applicable matrices described in scope. Updated mnemonic to match the mnemonic listed on the NIMS certificate of analysis.

EXHIBIT D

COVANCE
THE DEVELOPMENT SERVICES COMPANY

Covance Food Solutions

Method Validation Report

Mnemonics	BPA_LCMS
Method Title	Determination of BPA in Infant Formula, Commercial Packaged Non-Alcoholic Beverages and Food Packaging by LC-MS/MS
Validation Location	☐ Battle Creek ☐ Greenfield ☒ Madison ☐ Singapore ☐ Other: _____
Validation Reason	☒ New Method ☐ Method Equivalency ☐ Method Modification ☐ Other: _____
Protocol Identification (if applicable)	_____

DOCUMENT HISTORY:

Version #	Description	Date Issued
1	Original	01 SEP 2017

TABLE OF CONTENTS

SIGNATURES

This method validation was conducted in compliance with NA-FDA. 152.

This method is approved for the intended use as described in this report.

Written by: _____ 2017-Aug-18

Siheng Li
Staff Scientist
Covance Food Solutions

Date

Approved by: _____ 18 Aug 2017

Brent A. Rozema
Associate Director – Analyte Lead
Covance Food Solutions

Date

COVANCE KEY PERSONNEL

Role	Name
Method Developer/Analyst	Siheng Li
Analyst	Pamela Noack
Analyst	Lara Frankson
Technical Review	Jane Sabbatini

1 INTRODUCTION

1.1 Purpose

This is a validation study for a quantification method of bisphenol-A (BPA) in infant formula, food simulating liquids in packaging migration studies, and commercially packaged ready-to-consume beverages. The structure of BPA is shown in Figure 1. Historically, BPA in infant formula is analyzed by a client specific method (AN_BPA). This previous method involved the use of ion-pairing reagent in the chromatography separation, resulting in instrumental incompatibility with other LCMS-based assays. This is being replaced by a new method that uses 1 mM ammonium fluoride in water/acetonitrile as the mobile phase. Moreover, the old method employed SPE sample clean-up, introducing a potential source of sample contamination. In the new method, acetonitrile salting-out is used for BPA extraction in place of the SPE clean-up.

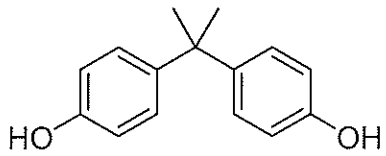

Figure 1. Structure of BPA

1.2 Scope

The method is applicable to the determination of BPA in powder/ready-to-feed infant formula, food simulants in packaging migration studies, and commercially packaged ready to consume non-alcoholic beverages. The validation evaluated specificity, linearity, accuracy, precision, and stability. Lacking certified reference materials of the appropriate matrices, the validation study assessed accuracy and precision using spikes of the study matrices.

1.3 Method

Covance developed method

1.3.1 Method Description

BPA extraction is required to remove the sample matrix in infant formula and beverages samples. As the first step, powder samples are reconstituted in water and liquid samples are used directly. Stable-isotope labeled BPA internal standard is incorporated prior to the sample extraction to correct for instrument response and losses during sample preparation. The samples are extracted with 1% acetic acid in acetonitrile. Sodium chloride is added to salt-out the BPA into the acetonitrile phase. The acetonitrile extract is kept at low temperature for removing the co-extracted lipids. The food simulating liquids are mixed with internal standard for the direct analysis. Analysis is performed by high pressure

liquid chromatography (HPLC) coupled to tandem quadrupole mass spectrometry (MS/MS). The MS/MS is configured to monitor precursor-fragment ion pairs in negative ESI mode for BPA and the internal standard.

1.3.2 Supporting Reference to help generate this method

Shi, Z., Fu, H., Xu, D. Huai, Q., Zhang, H., "Salting-Out Assisted Liquid/Liquid Extraction Coupled with Low-Temperature Purification for Analysis of Endocrine-Disrupting Chemicals in Milk and Infant Formula by Ultra High Performance Liquid Chromatography-Tandem Mass Spectrometry," *Food Analytical Methods*, *10* (5): 1523-1534 (2017)

2 MAJOR COMPUTER SYSTEMS

The major computer systems used on this study included, but not limited to, the following systems:

- MassHunter (Agilent)
- NIMS, Labware (Laboratory Information Management System)

3 STUDY MATERIALS AND REFERENCE SUBSTANCE

Study Materials
Identification: Infant Formula, Powder
Covance Sample Number: 4040974
Storage Condition: Room Temperature

Identification: Infant Formula, Ready-to-feed
Covance Sample Number: 6210937
Storage Condition: Refrigerated (5 ±3°C)

Identification: Food simulant A, ethanol 10% (v/v)
Covance Sample Number: 6249333
Storage Condition: Room Temperature

Identification: Food simulant B, 3% acetic acid (w/v)
Covance Sample Number: 6249334
Storage Condition: Room Temperature

Identification: Food simulant C, ethanol 20% (v/v)
Covance Sample Number: 6249335
Storage Condition: Room Temperature

Identification: Food simulant D1, ethanol 50% (v/v)
Covance Sample Number: 6249336
Storage Condition: Room Temperature

Identification: Food simulant D2, ethanol 95% (v/v)
Covance Sample Number: 6249337
Storage Condition: Room Temperature

Identification: Carbonated soft drink, regular (full calorie)
Covance Sample Number: 6348687
Storage Condition: Refrigerated (5 ±3°C)

Identification: Carbonated soft drink, diet, caffeine free
Covance Sample Number: 6348846
Storage Condition: Refrigerated (5 ±3°C)

Identification: 100% orange juice, with pulp
Covance Sample Number: 6348869
Storage Condition: Refrigerated (5 ±3°C)

Identification: Mango-flavored fruit juice
Covance Sample Number: 6348860
Storage Condition: Refrigerated (5 ±3°C)

Identification: Green tea beverage
Covance Sample Number: 6348942
Storage Condition: Refrigerated (5 ±3°C)

Identification: Dairy-based coffee, Frappuccino drink
Covance Sample Number: 6348920
Storage Condition: Refrigerated (5 ±3°C)

Identification: Sports drink, berry-flavored
Covance Sample Number: 6348957
Storage Condition: Refrigerated (5 ±3°C)

Identification: Energy drink, high-caffeine
Covance Sample Number: 6348924
Storage Condition: Refrigerated (5 ±3°C)

Identification: Grain-based beverage, yellow pea-based milk, unsweetened
Covance Sample Number: 6348874
Storage Condition: Refrigerated (5 ±3°C)

Identification: Meal replacement beverage, chocolate-flavored
Covance Sample Number: 6348907
Storage Condition: Refrigerated (5 ±3°C)

Reference Material
Identification: Bisphenol A standard
Supplier: Sigma-Aldrich, Inc.; catalog #239658; Lot # MKBX9458V; HPLC Purity: 100.0%, CAS: 80-05-7

Identification: Bisphenol A-d_{16} internal standard
Supplier: Cambridge Isotope Laboratories, Inc., catalog # DLM-1839-1; Lot # PR-21521; Purity: 99.3%

4 METHOD VALIDATION

4.1 Method Validation Criteria

Calibration curves:
- $r^2 > 0.995$
- Calibration curve residuals ≤15% for WS1 and ≤10% for other calibration standards

QC spikes for infant formula and food simulants:
- Recovery of 70 – 125%
- %RSD ≤ 15%

Taken from "Appendix K, Guidelines for Dietary Supplements and Botanicals, AOAC Official Methods of Analysis, AOAC International, 19th edition, Gaithersburg, MD (2012)". See Appendix III.

QC spikes for commercially packaged beverage:
- Recovery: 60 – 140% (< 2 µg/L); 80 – 120% (2 – 20 µg/L)
- %RSD: ≤ 20% (< 2 µg/L); ≤ 10% (2 – 5 µg/L); ≤ 5% (5 – 20 µg/L)

Taken from "AOAC SMPR draft (version 7; July 11, 2017) – Determination of free Bisphenol A (BPA) in commercially packaged ready to consume carbonated and non-carbonated water and non-alcoholic beverages". See Appendix III.

Limit of Quantification (LOQ):
- 0.60 ng/g (ppb) in water/ethanol food simulant
- 0.30 ng/g (ppb) in ready-to-feed infant formula
- 2.0 ng/g (ppb) in powdered infant formula
- 0.3 µg/L in commercially packaged ready-to-consume beverage

4.2 Specificity

MRM (multiple reaction monitoring) was utilized for BPA detection. The target analyte is identified from the matrix interference by evaluating the ratio of their relative intensities between different mass transitions. Specificity was evaluated by analyzing the representative matrices and the matrices fortified with BPA to demonstrate the proper identification of BPA. In coffee samples, the accurate integration of m/z 227.0>211.9 peak was impacted by an interference peak, resulting in the use of m/z 227.0>133.0 transition for quantitation in this matrix type. Figure 2 shows an example of the extracted-ion chromatograms of the matrices spiked at LOQ level.

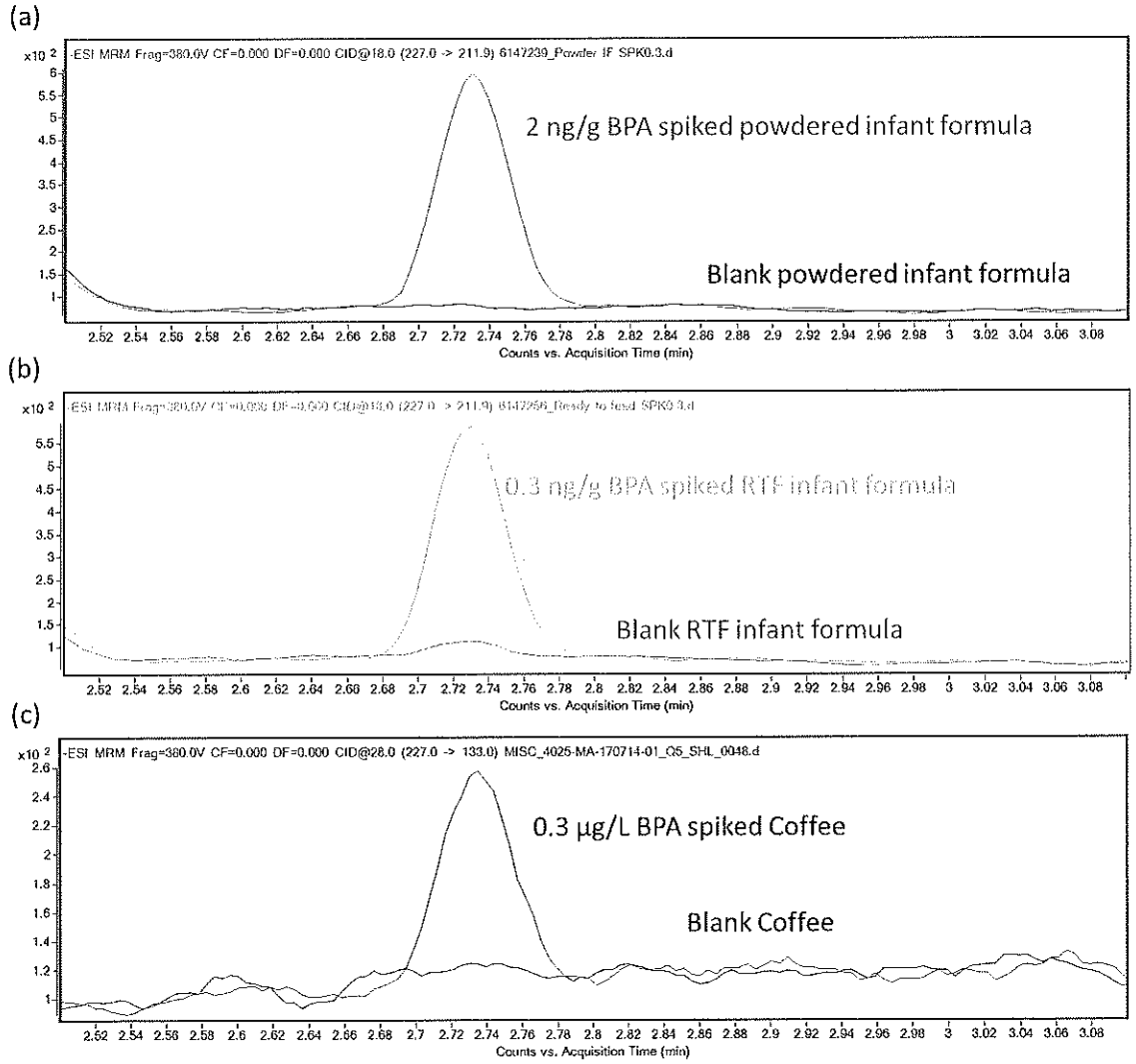

Figure 2. The overlay of the extracted ion chromatograms of the blank and spiked powdered infant formula, at 2.0 ng/g spike level (a); the blank and spiked ready-to-feed infant formula, at 0.3 ng/g spike level (b); the blank and spiked coffee, at 0.3 µg/L spike level (c).

4.3 Range

The validated range for the determination of BPA in various matrices is shown in table 1. The lower end of each range is considered the limit of quantification (LOQ).

Table 1. Quantification range of BPA in matrix

Matrix	
Food simulant (water ethanol mixture) (ng/g)	0.6 – 20
Powdered IF (ng/g)	2.0 – 133
Ready-to-feed IF (ng/g)	0.3 – 20
Beverage (µg/L or ng/g)	0.3 – 20

4.4 Linearity

To evaluate the linearity of the BPA calibration curve, BPA working standard solutions containing internal standard (BPA-d_{16}) were prepared at 0.10, 0.20, 1.00, 2.00, 10.0, 20.0 and 30.0 ng/mL. The linearity was determined by using a linear internal calibration with 1/x weighting of the response factor. The calibration curve residuals were ≤15% for WS1 and ≤10% for other calibration standards. The coefficients of determination (r^2) for BPA quantification transition were all >0.998. No more than 10% of the total number of working standard calibration points can be excluded from a calibration curve due to atypical instrument response or injection error. The statistical data of the calibration curve residuals and r^2 from different analysis dates were summarized in Table 2. A representative calibration curve is presented in Appendix I.

Table 2. Calibration Curve Residuals

BPA Std	Concentration (ng/mL)	Day 1 (%)	Day 2 (%)	Day 3 (%)	Day 4 (%)	Day 5 (%)
WS1-1	0.1	-6.7	-	-1.3	-15.0	-
WS1-2	0.1	9.8	1.9	-9.9	-9.5	13.7
WS2-1	0.2	0.1	-2.5	2.7	2.2	-1.9
WS2-2	0.2	4.0	-2.7	-2.1	9.2	3.0
WS3-1	1.0	-8.6	1.3	6.6	2.0	-0.7
WS3-2	1.0	2.1	-8.4	0.4	3.0	-6.6
WS4-1	2.0	-3.0	-3.7	0.4	2.2	-5.0
WS4-2	2.0	-8.1	-9.0	3.1	3.3	-1.5
WS5-1	10.0	1.4	-1.3	1.5	6.9	-0.8
WS5-2	10.0	-0.6	-2.6	-	-1.8	-2.0
WS6-1	20.0	1.0	-0.4	-0.5	-3.6	4.9
WS6-2	20.0	-	3.9	-0.9	0.3	-4.2
WS7-1	30.0	NI	NI	NI	NI	4.0
WS7-2	30.0	NI	NI	NI	NI	-2.9
r^2, *m/z* 227.0>211.9		0.9993	0.9989	0.9998	0.9986	0.9985

"-" Data were excluded from calibration curve due to the residuals out of range.
NI. The level of working standard was not injected in the analysis of the sample batch.

4.5 Accuracy and Precision

Accuracy was determined by spiking the matrices at three concentration levels covering the target analytical range. Three replicates were analyzed for each concentration level and matrix by two different analysts on 3 different days, resulting in 9 overall replicates for each concentration level and matrix combination. Each analytical run assayed one replicate of the unspiked material to demonstrate absence of the incurred analyte (<LOQ). The sample spikes were prepared by adding an appropriate volume of the BPA spiking solution (BPA-IWS1 or BPA-IWS2) prior to extraction. The detailed results are presented in appendix II.

Table 3. Overall mean BPA recovery in infant formula at each spiking level obtained by two different analysts on 3 different days (n = 9)

Matrix	Overall Mean Recovery (%)		
Powdered infant formula	**2.0 ng/g**	**10.0 ng/g**	**133.3 ng/g**
	100.5	103.6	102.2
Ready-to-feed infant formula	**0.3 ng/g**	**1.5 ng/g**	**20 ng/g**
	102.8	98.2	95.7

Table 4. Overall mean BPA recovery in food simulants at each spiking level obtained by two different analysts on 3 different days (n = 9)

Matrix	Overall Mean Recovery (%)		
	0.6 ng/g	**1.5 ng/g**	**20 ng/g**
Water	99.7	97.8	100.7
10% ethanol	100.2	99.1	99.9
3% acetic acid	99.1	100.0	102.8
20% ethanol	98.9	102.9	102.6
50% ethanol	103.2	103.8	102.8
95% ethanol	105.8	104.2	100.7

Table 5. Overall mean BPA recovery in each beverage matrix at each spiking level obtained by two different analysts on 3 different days (n = 9)

Matrix	Overall Mean Recovery (%)		
	0.3 µg/L	1.5 µg/L	20 µg/L
Regular carbonated soft drink	95.6	102.0	99.8
Diet carbonated soft drink	92.4	96.8	100.8
Juice, with pulp	95.0	98.5	102.5
Juice, without pulp	93.1	98.1	96.9
Tea	99.1	101.9	98.9
Dairy-based coffee drink	98.6	102.4	100.5
Sports drink	95.7	101.0	101.3
Energy drink	126.4	103.4	99.1
Grain-based beverage	103.9	98.0	100.3
Meal replacement beverage	108.5	105.4	103.4

Precision were evaluated based on the recovery results generated in the accuracy experiment described in the above section. Detailed results including repeatability precision are provided in Appendix II. Table 6 summarizes the obtained intermediate precision (%RSD_i) results at the LOQ level. The precision was excellent with all values well below 20 %RSD.

Table 6. Intermediate precision (%RSD_i, n = 9) obtained at LOQ spiking level.

Matrix	Levels	%RSD_{INT}
Powder infant formula	**2 ng/g**	9.5
Ready to feed infant formula	**0.3 ng/g**	6.3
Food simulants	**0.6 ng/g**	
water		5.4
10% ethanol		5.8
3% acetic acid		4.8
20% ethanol		3.1
50% ethanol		4.4
95% ethanol		4.5
Beverages	**0.3 µg/L**	
Regular carbonated soft drink		7.5
Diet carbonated soft drink		7.5
Juice, with pulp		11.1
Juice, without pulp		3.0
Tea		6.0
Dairy-based coffee drink		10.1
Sports drink		5.7
Energy drink		7.3
Grain-based beverage		11.1
Meal replacement beverage		8.2

4.6 Stability of Reference and Sample Solutions

The stability of the BPA and BPA-d16 stock solution was tested and confirmed as 3 months stability by comparing the analyte/IS area ratio between the 10 ng/mL calibration standard prepared by the 3-months old stock solution and the freshly prepared stock solution. The BPA stock solution (200 µg/mL and 100 ng/mL) and BPA-d16 stock solution (200 µg/mL and 1 µg/mL) were kept refrigerated (5 ± 2 °C) for the 3 month period until the test. A fresh BPA stock solution (200 µg/mL and 100 ng/mL) and BPA-d16 stock solution (200 µg/mL and 1 µg/mL) were prepared from the new weighting of powder standards. The 3-months old BPA stock solution was used to prepare the 10 ng/mL calibration solution with fresh IS stock solution, and the 3-months old BPA-d16 stock solution was used to prepare the 10 ng/mL calibration solution with fresh BPA stock solution. Three replicates of each 10 ng/mL solution were analyzed and the mean standard/IS area ratio are shown in Table 7.

Table 7. Stability (expressed as relative response of old *vs.* new standard) of BPA and BPA-d16 the stock solution stored for 3 months refrigerated

BPA stock solution	IS stock solution	BPA conc. (ng/mL)	Mean Std/ IS area ratio	% relative response
Fresh BPA, 200 µg/mL	Fresh BPA-d16, 200 µg/mL	10	13.48	
3 mo. BPA, 200 µg/mL	Fresh BPA-d16, 200 µg/mL	10	14.81	109.87
3 mo.BPA, 100 ng/mL	Fresh BPA-d16, 200 µg/mL	10	12.39	91.96
Fresh BPA, 200 µg/mL	3-mo. BPA-d16, 200 µg/mL	10	13.54	100.44
Fresh BPA, 200 µg/mL	3-mo. BPA-d16, 1 µg/mL	10	13.17	97.70

Working standard (Level 1-6) refrigerated stability was tested at 3 days and 7 days (see Table 8). The standards used for comparison were from a freshly prepared intermediate standard from that day. The % change in the results at 3 days and 7 days old vs. freshly prepared working standards was <10%, with the exception of 7 days old WS-1.

Table 8. Stability (expressed as relative response of old *vs.* new standard) of BPA in working standards (Level 1-6) stored for up to 1 week refrigerated

BPA Std ID	Concentration (ng/mL)	Fresh	3 days		Fresh	7 days	
		Std/IS Area Ratio	Std/IS Area Ratio	%Relative Response	Std/IS Area Ratio	Std/IS Area Ratio	%Relative Response
WS1	0.1	0.128	0.131	102.3	0.141	0.164	116.3
WS2	0.2	0.291	0.278	95.5	0.268	0.262	97.8
WS3	1	1.346	1.346	100.0	1.267	1.321	104.3
WS4	2	2.407	2.462	102.3	2.354	2.431	103.3
WS5	10	12.731	12.833	100.8	13.192	13.258	100.5
WS6	20	24.691	26.132	105.8	24.656	24.632	99.9

Stability of sample extracts was tested at the seven day time point. Triplicate of the same spiked sample extracts (0.3 ng/g for infant formula extract and 0.6 ng/g for food simulants) were tested at 0 and 7 day time point by quantification against the original set of working standards prepared with the samples. The samples were stored refrigerated between the two testing time points. The measured concentration was shown in Table 9. The stability of the working standards has been set at 7 days. In addition, even though not tested, the intermediate standard solutions which are in the same solvent are also set at 7 days.

Table 9. Comparison of BPA calculated concentrations in the same sample extract freshly prepared *vs.* stored refrigerated for 7 days

Experiment	Replicate	Concentration (ng/g)		
		Infant formula extract	10% ethanol	95% ethanol
Freshly prepared	1	0.322	0.622	0.581
	2	0.290	0.561	0.613
	3	0.295	0.569	0.620
	Mean	0.302	0.584	0.605
	RSD (%)	5.6	5.7	3.4
7 days	1	0.315	0.634	0.619
	2	0.318	0.604	0.603
	3	0.332	0.541	0.575
	Mean	0.322	0.593	0.599
	RSD (%)	2.8	7.9	3.7
	% Change	6.6	1.5	-1.0

In conclusion, the working standard solutions are stable for one week when stored refrigerated. The sample extracts can be stored for up to 7 days refrigerated prior to the LC-MS/MS analysis when quantitated against the original working standards prepared at the same time.

4.7 Limit of Quantification

The LOQ for each analyte is listed in section 4.3. Adequate precision and accuracy at these levels for BPA was demonstrated during validation as presented in this report. Standard addition can be employed as necessary with matrices that do not perform acceptably using solvent standards with the stable-labeled isotope internal standard.

5 MEASUREMENT UNCERTAINTY

Measurement uncertainty of BPA was determined based on the precision data shown in Table 6 (LOQ).

Table 10. Measurement uncertainty

Matrix	n	RSD (%)	Coverage Factor 95% Confidence	Measurement Uncertainty (%)
Powdered infant formula	9	9.5	2.306	21.9
Ready-to-feed infant formula	9	6.3	2.306	14.5
Water	9	5.4	2.306	12.5
10% Ethanol	9	5.8	2.306	13.4
3% Acetic Acid	9	4.8	2.306	11.1
20% Ethanol	9	3.1	2.306	7.1
50% Ethanol	9	4.4	2.306	10.1
95% Ethanol	9	4.5	2.306	10.4
Regular carbonated soft drink	9	7.5	2.306	17.3
Diet carbonated soft drink	9	7.5	2.306	17.4
Juice, with pulp	9	11.1	2.306	25.5
Juice, without pulp	9	3.0	2.306	7.0
Tea	9	6.0	2.306	13.9
Dairy-based coffee drink	9	11.3	2.306	26.1
Sport drink	9	5.7	2.306	13.1
Energy drink	9	7.3	2.306	16.7
Grain-based beverage	9	11.1	2.306	25.6
Meal replacement beverage	9	8.2	2.306	18.9

6 RAW DATA

The instrument raw data was collected to the network drive with the following data path:

\\msnsan03\chemistry\mass_spec_data\ncfs\ MassHunter\BPA\2017_Validation\

The paper data to support days of analysis will be archived according to the appropriate SOP in the department central files, or attached to NIMS batches, as appropriate. Standard and solution preparations were also performed in NIMS or attached to batches in NIMS.

Table 11 - Data Folder Locations

Data Folder Name	NIMS Batch ID
20170519_Validation Day 1	MISC_4025-MA-170515-1
20170521_Validation Day 2	MISC_4025-MA-170520-1
20170522_Validation	MISC_4025-MA-170522-2
20170525_Validation Day 3	MISC_4025-MA-170524-1
20170531_Validation Day 4	MISC_4025-MA-170531-1
20170619_Food Simulant Validation	MISC_4025-MA-170619-1
20170619_Food Simulant Validation_2	MISC_4025-MA-170619-2
20170622_Food Simulant Validation_3	MISC_4025-MA-170619-3
20170610_Beverage Validation	MISC_4025-MA-170607-1
20170613_Beverage Validation	MISC_4025-MA-170612-1
20170614_Beverage Validation	MISC_4025-MA-170614-2
20170703_Beverage Validation	MISC_4025-MA-170703-1
20170610_Beverage Validation	MISC_4025-MA-170707-1
20170613_Beverage Validation	MISC_4025-MA-170610-2
20170714_Beverage Validation	MISC_4025-MA-170714-1
20170717_Beverage Validation	MISC_4025-MA-170717-1

7 WRITTEN METHOD ASSOCIATED WITH THIS VALIDATION

Method name, BPA_LCMS
Effective date, 01 Sep 2017
Version number. 1

8 CONCLUSION

Based on the reported testing results, the BPA_LCMS method met or exceeded the validation criteria for specificity, linearity, accuracy, and precision for the determination of BPA in powdered and ready to feed infant formula, beverages and food simulants.

APPENDIX I

Example Calibration Curves

Data from NIMS batch: MISC_4025-MA-170520-1

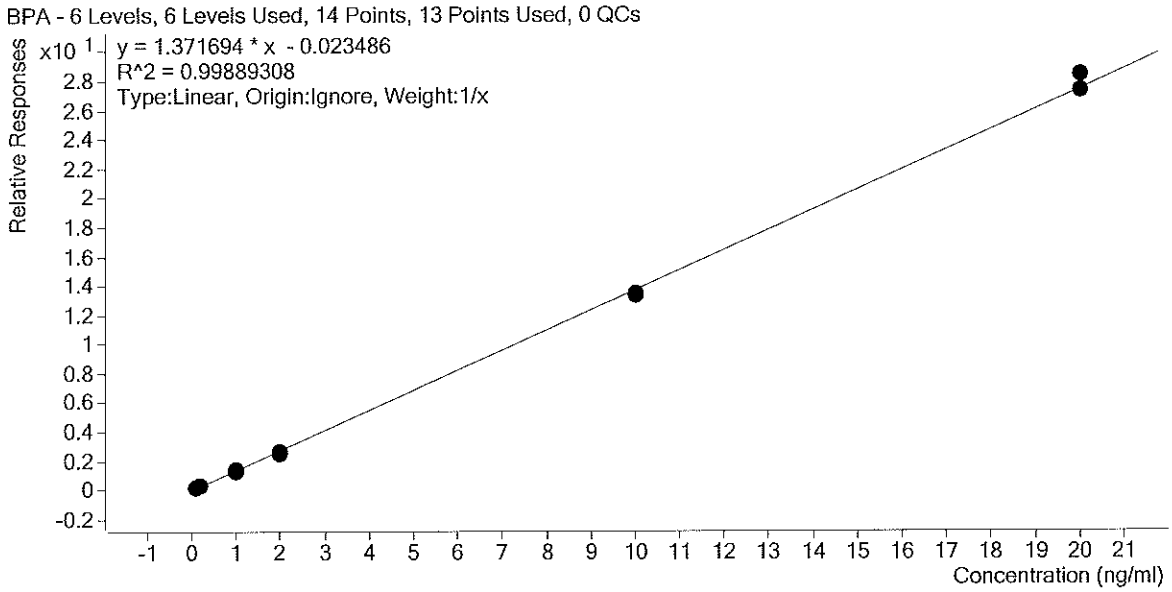

Figure AI-1 BPA calibration curve

APPENDIX II Recoveries of BPA in matrices.

Accuracy (% recovery), repeatability precision (%RSDr) and intermediate precision (%RSDi) results obtained at three spiking levels by two analysts on three days for the evaluated matrices

Matrix	Table
Powdered infant formula	AII-1
Ready-to-feed infant formula	AII-2
Water	AII-3
Food simulant A	AII-4
Food simulant B	AII-5
Food simulant C	AII-6
Food simulant D1	AII-7
Food simulant D2	AII-8
Regular carbonated soft drink	AII-9
Diet carbonated soft drink	AII-10
Juice, with pulp	AII-11
Juice, without pulp	AII-12
Tea	AII-13
Dairy-based coffee drink	AII-14
Sports drink	AII-15
Energy drink	AII-16
Grain-based beverage	AII-17
Meal replacement beverage	AII-18

Table AII-1. powdered infant formula

Day/Analyst	Replicate	BPA Concentration (ng/g)			BPA Recovery (%)		
		2.0	10.0	133	2.0 ng/g	10.0 ng/g	133 ng/g
Day 1/ Analyst 1	1	2.26	10.3	150	113.2	102.8	112.4
	2	2.01	9.95	132	100.5	99.5	98.6
	3	2.06	10.2	131	102.9	102.3	98.5
	Mean				105.5	101.5	103.2
	Standard Deviation				6.75	1.78	8.00
	%RSDr				6.4	1.8	7.8
Day 2/ Analyst 1	1	2.25	10.9	141	112.5	109.5	105.6
	2	2.09	11.0	136	104.6	109.7	101.6
	3	2.05	10.5	145	102.3	104.6	108.9
	Mean				106.5	107.9	105.4
	Standard Deviation				5.35	2.89	3.66
	%RSDr				5.0	2.7	3.5
Day 3/ Analyst 2	1	1.72	10.2	125	86.0	102.4	94.1
	2	1.76	9.59	131	87.8	95.9	98.3
	3	1.90	10.5	135	95.1	105.3	101.4
	Mean				89.6	101.2	97.9
	Standard Deviation				4.82	4.81	3.66
	%RSDr				5.4	4.8	3.7
Overall	**Mean**				**100.5**	**103.6**	**102.2**
	Standard Deviation				**9.56**	**4.41**	**5.80**
	%RSDi				**9.5**	**4.3**	**5.7**

Table AII-2. ready-to-feed infant formula

Day/Analyst	Replicate	BPA Concentration (ng/g)			BPA Recovery (%)		
		0.30	1.50	20.0	0.30 ng/g	1.50 ng/g	20.0 ng/g
Day 1/ Analyst 1	1	0.33	1.48	18.7	111.1	98.8	93.6
	2	0.29	1.55	20.1	96.8	103.5	100.6
	3	0.28	1.43	20.6	93.5	95.2	103.0
				Mean	100.5	99.2	99.1
				Standard Deviation	9.36	4.16	4.88
				%RSDr	9.3	4.2	4.9
Day 2/ Analyst 1	1	0.32	1.48	18.8	106.9	100.0	95.1
	2	0.32	1.47	19.1	106.7	98.8	96.6
	3	0.33	1.48	18.3	109.1	99.9	92.9
				Mean	107.6	99.6	94.9
				Standard Deviation	1.33	0.67	1.86
				%RSDr	1.2	0.7	2.0
Day 3/ Analyst 2	1	0.32	1.44	18.3	107.2	95.8	91.5
	2	0.29	1.45	18.3	95.7	96.3	91.6
	3	0.30	1.44	19.3	98.5	95.9	96.5
				Mean	100.5	96.0	93.2
				Standard Deviation	6.0	0.3	3.1
				%RSDr	6.0	0.3	3.1
Overall				**Mean**	**102.8**	**98.2**	**95.7**
				Standard Deviation	**6.63**	**2.71**	**3.97**
				%RSDi	**6.3**	**2.6**	**4.3**

Table AII-3. water

Day/Analyst	Replicate	BPA Concentration (ng/g)			BPA Recovery (%)		
		0.60	1.50	20.0	0.60 ng/g	1.50 ng/g	20.0 ng/g
Day 1/ Analyst 1	1	0.65	1.45	19.7	108.7	96.8	98.7
	2	0.59	1.35	20.8	98.2	90.0	103.8
	3	0.61	1.55	21.8	101.2	103.6	109.2
				Mean	102.7	96.8	103.9
				Standard Deviation	5.41	6.80	5.25
				%RSDr	5.3	7.0	5.1
Day 2/ Analyst 2	1	0.59	1.36	18.2	98.2	91.0	91.1
	2	0.57	1.43	19.9	95.4	95.4	99.7
	3	0.55	1.51	21.3	92.3	100.8	106.3
				Mean	95.3	95.7	99.0
				Standard Deviation	2.95	4.91	7.62
				%RSDr	3.1	5.1	7.7
Day 3/ Analyst 2	1	0.59	1.61	19.4	98.8	107.6	97.1
	2	0.64	1.46	19.9	107.5	97.6	99.3
	3	0.58	1.46	20.3	96.6	97.0	101.3
				Mean	101.0	100.7	99.2
				Standard Deviation	5.76	5.95	2.10
				%RSDr	5.7	5.9	2.1
Overall				**Mean**	**99.7**	**97.8**	**100.7**
				Standard Deviation	**5.39**	**5.63**	**5.31**
				%RSDi	**5.4**	**5.8**	**5.3**

Table AII-4. Food simulant A (10% ethanol)

Day/Analyst	Replicate	BPA Concentration (ng/g)			BPA Recovery (%)		
		0.6	1.5	20.0	0.6 ng/g	1.5 ng/g	20 ng/g
Day 1/ Analyst 1	1	0.65	1.49	18.7	108.3	99.5	93.3
	2	0.61	1.47	20.2	102.1	97.7	100.8
	3	0.59	1.48	18.4	98.3	98.4	92.1
				Mean	102.9	98.5	95.4
				Standard Deviation	5.05	0.91	4.71
				%RSDr	4.9	0.9	4.9
Day 2/ Analyst 2	1	0.53	1.42	20.0	88.5	94.9	99.9
	2	0.60	1.51	21.4	100.5	100.9	107.2
	3	0.59	1.39	20.9	98.8	92.9	104.4
				Mean	95.9	96.2	103.8
				Standard Deviation	6.49	4.16	3.68
				%RSDr	6.8	4.3	3.5
Day 3/ Analyst 2	1	0.64	1.54	20.7	107.3	102.6	103.4
	2	0.59	1.49	19.2	98.5	99.3	95.9
	3	0.59	1.59	20.5	99.1	105.8	102.3
				Mean	101.6	102.6	100.5
				Standard Deviation	4.92	3.25	4.05
				%RSDr	4.9	3.2	4.0
Overall				**Mean**	**100.2**	**99.1**	**99.9**
				Standard Deviation	**5.77**	**3.86**	**5.16**
				%RSDi	**5.8**	**3.9**	**5.1**

Table AII-5. Food simulant B (3% acetic acid in water)

Day/Analyst	Replicate	BPA Concentration (ng/g)			BPA Recovery (%)		
		0.60	1.50	20.0	0.60 ng/g	1.50 ng/g	20 ng/g
Day 1/ Analyst 1	1	0.64	1.54	19.6	106.6	102.6	97.9
	2	0.57	1.43	19.9	95.0	95.0	99.6
	3	0.59	1.55	20.7	98.9	103.1	103.5
				Mean	100.2	100.2	100.3
				Standard Deviation	5.88	4.52	2.88
				%RSDr	5.9	4.5	2.9
Day 2/ Analyst 2	1	0.64	1.54	20.2	106.1	102.9	100.9
	2	0.61	1.50	20.9	101.2	100.2	104.6
	3	0.59	1.44	20.8	98.7	96.3	104.1
				Mean	102.0	99.8	103.2
				Standard Deviation	3.76	3.32	2.01
				%RSDr	3.7	3.3	1.9
Day 3/ Analyst 2	1	0.56	1.51	20.8	94.2	100.6	104.1
	2	0.57	1.48	20.9	94.2	98.6	104.6
	3	0.58	1.51	21.1	96.7	100.7	105.5
				Mean	95.0	100.0	104.7
				Standard Deviation	1.44	1.18	0.71
				%RSDr	1.5	1.2	0.7
Overall				**Mean**	**99.1**	**100.0**	**102.8**
				Standard Deviation	**4.75**	**2.88**	**2.63**
				%RSDi	**4.8**	**2.9**	**2.6**

Table AII-6. Food simulant C (20% ethanol)

Day/Analyst	Replicate	BPA Concentration (ng/g)			BPA Recovery (%)		
		0.6	1.5	20.0	0.6 ng/g	1.5 ng/g	20 ng/g
Day 1/ Analyst 1	1	0.56	1.66	18.9	94.1	110.8	94.7
	2	0.58	1.49	19.3	97.2	99.5	96.6
	3	0.59	1.51	21.4	98.5	100.6	107.0
				Mean	96.6	103.6	99.4
				Standard Deviation	2.26	6.23	6.62
				%RSDr	2.3	6.0	6.7
Day 2/ Analyst 2	1	0.61	1.48	19.9	101.9	98.7	99.3
	2	0.60	1.66	20.4	100.4	110.5	102.2
	3	0.60	1.51	20.6	100.5	100.9	102.8
				Mean	100.9	103.4	101.4
				Standard Deviation	0.84	6.27	1.87
				%RSDr	0.8	6.1	1.8
Day 3/ Analyst 2	1	0.58	1.54	21.1	96.9	102.4	105.7
	2	0.62	1.49	21.5	103.9	99.4	107.3
	3	0.58	1.55	21.6	96.7	103.4	108.0
				Mean	99.2	101.7	107.0
				Standard Deviation	4.10	2.08	1.18
				%RSDr	4.1	2.0	1.1
Overall				**Mean**	**98.9**	**102.9**	**102.6**
				Standard Deviation	**3.04**	**4.63**	**4.87**
				%RSDi	**3.1**	**4.5**	**4.8**

Table AII-7. Food simulant D1 (50% ethanol)

Day/Analyst	Replicate	BPA Concentration (ng/g)			BPA Recovery (%)		
		0.6	1.5	20.0	0.6 ng/g	1.5 ng/g	20 ng/g
Day 1/ Analyst 1	1	0.58	1.58	20.8	97.5	105.5	104.2
	2	0.58	1.51	21.2	97.3	100.4	106.0
	3	0.67	1.50	20.9	111.1	99.9	104.3
				Mean	102.0	101.9	104.8
				Standard Deviation	7.91	3.10	1.01
				%RSDr	7.8	3.0	1.0
Day 2/ Analyst 2	1	0.62	1.71	21.3	103.0	114.1	106.5
	2	0.62	1.47	20.1	103.4	97.8	100.7
	3	0.64	1.51	20.6	106.3	100.9	103.0
				Mean	104.2	104.3	103.4
				Standard Deviation	1.80	8.66	2.92
				%RSDr	1.7	8.3	2.8
Day 3/ Analyst 2	1	0.60	1.50	20.2	99.9	100.1	101.0
	2	0.62	1.58	19.7	103.8	105.6	98.5
	3	0.64	1.64	20.1	106.8	109.5	100.6
				Mean	103.5	105.1	100.0
				Standard Deviation	3.46	4.72	1.34
				%RSDr	3.4	4.5	1.3
Overall				**Mean**	**103.2**	**103.8**	**102.8**
				Standard Deviation	**4.52**	**5.36**	**2.72**
				%RSDi	**4.4**	**5.1**	**2.6**

Table AII-8. Food simulant D2 (95% ethanol)

Day/Analyst	Replicate	BPA Concentration (ng/g)			BPA Recovery (%)		
		0.6	1.5	20.0	0.6 ng/g	1.5 ng/g	20 ng/g
Day 1/ Analyst 1	1	0.65	1.54	19.7	108.1	102.4	98.3
	2	0.62	1.64	19.7	102.7	109.1	98.7
	3	0.66	1.59	21.8	110.8	105.9	109.0
				Mean	107.2	105.8	102.0
				Standard Deviation	4.12	3.35	6.07
				%RSDr	3.9	3.2	6.0
Day 2/ Analyst 2	1	0.62	1.67	19.7	103.8	111.0	98.6
	2	0.66	1.50	19.9	109.9	100.3	99.4
	3	0.63	1.53	19.6	104.5	102.1	97.9
				Mean	106.1	104.5	98.6
				Standard Deviation	3.34	5.73	0.75
				%RSDr	3.2	5.5	0.8
Day 3/ Analyst 2	1	0.67	1.50	21.1	110.9	100.3	105.4
	2	0.58	1.63	19.8	96.4	108.8	99.0
	3	0.63	1.47	20.1	105.4	98.1	100.4
				Mean	104.2	102.4	101.6
				Standard Deviation	7.32	5.65	3.36
				%RSDr	7.0	5.5	3.3
Overall				**Mean**	**105.8**	**104.2**	**100.7**
				Standard Deviation	**4.70**	**4.60**	**3.83**
				%RSDi	**4.5**	**4.4**	**3.8**

Table AII-9. Regular carbonated soft drink

Day/Analyst	Replicate	BPA Concentration (µg/L)			BPA Recovery (%)		
		0.3	1.5	20	0.3 µg/L	1.5 µg/L	20 µg/L
Day 1/ Analyst 1	1	0.27	1.52	19.7	91.1	101.4	98.4
	2	0.27	1.54	20.0	91.4	102.6	99.8
	3	0.26	1.53	21.3	85.2	102.1	106.7
				Mean	89.2	102.0	101.6
				Standard Deviation	3.50	0.60	4.44
				$\%RSD_r$	3.9	0.6	4.4
Day 2/ Analyst 1	1	0.26	1.52	19.7	88.3	101.0	98.5
	2	0.32	1.59	19.6	108.0	105.8	97.9
	3	0.30	1.48	19.5	99.7	98.8	97.6
				Mean	98.7	101.9	98.0
				Standard Deviation	9.89	3.58	0.46
				$\%RSD_r$	10.0	3.5	0.5
Day 3/ Analyst 2	1	0.30	1.50	20.6	99.2	100.0	103.0
	2	0.29	1.50	19.7	96.6	99.7	98.7
	3	0.30	1.60	19.5	100.6	106.6	97.4
				Mean	98.8	102.1	99.7
				Standard Deviation	2.03	3.90	2.93
				$\%RSD_r$	2.0	3.8	2.9
Overall				**Mean**	**95.6**	**102.0**	**99.8**
				Standard Deviation	**7.15**	**2.67**	**3.10**
				$\%RSD_i$	**7.5**	**2.6**	**3.1**

Table AII-10. Diet carbonated soft drink

Day/Analyst	Replicate	BPA Concentration (µg/L)			BPA Recovery (%)		
		0.3	1.5	20	0.3 µg/L	1.5 µg/L	20 µg/L
Day 1/ Analyst 1	1	0.27	1.45	20.2	90.3	96.4	101.1
	2	0.25	1.40	19.8	83.3	93.6	98.9
	3	0.24	1.42	19.4	79.7	94.5	97.2
				Mean	84.4	94.8	99.1
				Standard Deviation	5.39	1.43	1.96
				$\%RSD_r$	6.4	1.5	2.0
Day 2/ Analyst 2	1	0.28	1.48	21.1	91.9	98.6	105.7
	2	0.28	1.50	19.7	94.0	100.2	98.3
	3	0.30	1.53	21.2	100.1	101.8	106.2
				Mean	95.3	100.2	103.4
				Standard Deviation	4.26	1.60	4.42
				$\%RSD_r$	4.4	1.6	4.3
Day 3/ Analyst 2	1	0.29	1.40	19.5	95.5	93.4	97.4
	2	0.30	1.47	20.1	98.3	98.0	100.5
	3	0.29	1.42	20.3	98.1	94.5	101.6
				Mean	97.3	95.3	99.8
				Standard Deviation	1.56	2.44	2.19
				$\%RSD_r$	1.6	2.5	2.2
Overall				**Mean**	**92.4**	**96.8**	**100.8**
				Standard Deviation	**6.96**	**3.04**	**3.33**
				$\%RSD_i$	**7.5**	**3.2**	**3.3**

Table AII-11. Juice with pulp

Day/Analyst	Replicate	BPA Concentration (µg/L)			BPA Recovery (%)		
		0.3	1.5	20	0.3 µg/L	1.5 µg/L	20 µg/L
Day 1/ Analyst 1	1	0.23	1.41	21.6	76.5	93.9	108.1
	2	0.26	1.38	20.3	86.6	92.1	101.7
	3	0.26	1.45	21.0	86.0	96.4	104.9
				Mean	83.0	94.1	104.9
				Standard Deviation	5.67	2.16	3.20
				$\%RSD_r$	6.8	2.3	3.1
Day 2/ Analyst 2	1	0.30	1.49	20.9	98.6	99.6	104.4
	2	0.28	1.54	20.7	92.0	102.9	103.7
	3	0.30	1.57	20.0	100.3	104.5	99.8
				Mean	97.0	102.3	102.6
				Standard Deviation	4.38	2.50	2.48
				$\%RSD_r$	4.6	2.4	2.4
Day 3/ Analyst 2	1	0.30	1.50	19.9	100.3	99.8	99.4
	2	0.33	1.52	19.4	109.2	101.4	96.9
	3	0.32	1.43	20.8	105.5	95.5	103.9
				Mean	105.0	98.9	100.1
				Standard Deviation	4.45	3.06	3.54
				$\%RSD_r$	4.2	3.1	3.5
Overall				**Mean**	**95.0**	**98.5**	**102.5**
				Standard Deviation	**10.51**	**4.22**	**3.41**
				$\%RSD_i$	**11.1**	**4.3**	**3.3**

Table AII-12. Juice without pulp

Day/Analyst	Replicate	BPA Concentration (µg/L)			BPA Recovery (%)		
		0.3	1.5	20	0.3 µg/L	1.5 µg/L	20 µg/L
Day 1/ Analyst 1	1	0.29	1.44	20.8	97.2	95.9	104.1
	2	0.27	1.51	20.9	91.1	101.0	104.7
	3	0.28	1.46	19.5	94.5	97.5	97.4
				Mean	94.3	98.1	102.1
				Standard Deviation	3.06	2.61	4.05
				$\%RSD_r$	3.2	2.7	4.0
Day 2/ Analyst 1	1	0.28	1.40	18.2	93.2	93.2	90.9
	2	0.27	1.43	18.9	89.9	95.2	94.6
	3	0.29	1.53	19.4	97.4	102.0	96.9
				Mean	93.5	96.8	94.1
				Standard Deviation	3.76	4.61	3.03
				$\%RSD_r$	4.0	4.8	3.2
Day 3/ Analyst 2	1	0.27	1.49	19.0	91.2	99.4	95.1
	2	0.27	1.49	18.5	90.2	99.4	92.4
	3	0.28	1.48	19.2	93.2	99.0	96.2
				Mean	91.5	99.2	94.6
				Standard Deviation	1.53	0.23	1.96
				$\%RSD_r$	1.6	0.2	2.1
Overall				**Mean**	**93.1**	**98.1**	**96.9**
				Standard Deviation	**2.82**	**2.86**	**4.72**
				$\%RSD_i$	**3.0**	**2.9**	**4.9**

Table AII-13. Tea

Day/Analyst	Replicate	BPA Concentration (µg/L)			BPA Recovery (%)		
		0.3	1.5	20	0.3 µg/L	1.5 µg/L	20 µg/L
Day 1/ Analyst 1	1	0.27	1.62	19.7	91.1	108.3	98.7
	2	0.31	1.60	18.9	102.5	106.6	94.7
	3	0.27	1.44	19.9	90.9	96.0	99.3
	Mean				94.8	103.7	97.6
	Standard Deviation				6.64	6.67	2.50
	$\%RSD_r$				7.0	6.4	2.6
Day 2/ Analyst 1	1	0.33	1.59	20.8	108.9	106.2	104.0
	2	0.29	1.54	19.3	96.8	102.9	96.6
	3	0.31	1.47	19.3	104.4	98.0	96.6
	Mean				103.4	102.4	99.0
	Standard Deviation				6.12	4.13	4.27
	$\%RSD_r$				5.9	4.0	4.3
Day 3/ Analyst 2	1	0.30	1.43	20.9	98.5	95.2	104.4
	2	0.31	1.55	19.9	101.8	103.4	99.6
	3	0.29	1.50	19.2	97.1	99.9	95.8
	Mean				99.1	99.5	99.9
	Standard Deviation				2.41	4.11	4.31
	$\%RSD_r$				2.4	4.1	4.3
Overall	**Mean**				**99.1**	**101.9**	**98.9**
	Standard Deviation				**5.96**	**4.79**	**3.44**
	$\%RSD_i$				**6.0**	**4.7**	**3.5**

Table AII-14. Dairy-based coffee drink

Day/Analyst	Replicate	BPA Concentration (µg/L)			BPA Recovery (%)		
		0.3	1.5	20	0.3 µg/L	1.5 µg/L	20 µg/L
Day 1/ Analyst 1	1	0.31	1.63	20.9	104.3	108.9	104.3
	2	0.31	1.65	19.9	102.8	109.9	99.7
	3	0.32	1.53	20.4	107.2	102.1	102.0
				Mean	104.8	106.9	102.0
				Standard Deviation	2.24	4.24	2.30
				$\%RSD_r$	2.1	4.0	2.3
Day 2/ Analyst 2	1	0.27	1.59	19.9	89.7	106.1	99.5
	2	0.29	1.59	19.2	95.0	105.8	95.8
	3	0.24	1.41	19.7	80.5	94.2	98.5
				Mean	88.4	102.0	97.9
				Standard Deviation	7.34	6.79	1.91
				$\%RSD_r$	8.3	6.7	2.0
Day 3/ Analyst 2	1	0.34	1.45	19.6	113.2	96.4	97.8
	2	0.31	1.47	20.1	101.9	98.0	100.7
	3	0.28	1.51	21.2	93.1	100.6	105.8
				Mean	102.7	98.3	101.4
				Standard Deviation	10.08	2.12	4.05
				$\%RSD_r$	9.8	2.2	4.0
Overall				**Mean**	**98.6**	**102.4**	**100.5**
				Standard Deviation	**9.99**	**5.59**	**3.16**
				$\%RSD_i$	**10.1**	**5.5**	**3.1**

Table AII-15. Sports drink

Day/Analyst	Replicate	BPA Concentration (µg/L)			BPA Recovery (%)		
		0.3	1.5	20	0.3 µg/L	1.5 µg/L	20 µg/L
Day 1/ Analyst 1	1	0.28	1.52	20.2	92.2	101.0	100.9
	2	0.31	1.50	21.0	102.7	100.3	104.8
	3	0.28	1.59	21.3	92.6	105.9	106.5
				Mean	95.8	102.4	104.1
				Standard Deviation	5.95	3.05	2.87
				$\%RSD_r$	6.2	3.0	2.8
Day 2/ Analyst 1	1	0.26	1.43	21.2	88.0	95.1	106.0
	2	0.30	1.60	20.0	100.7	106.6	99.9
	3	0.27	1.60	19.6	91.1	106.6	98.1
				Mean	93.3	102.8	101.4
				Standard Deviation	6.62	6.64	4.14
				$\%RSD_r$	7.1	6.5	4.1
Day 3/ Analyst 2	1	0.29	1.43	19.9	98.1	95.5	99.6
	2	0.31	1.56	19.7	102.7	104.3	98.5
	3	0.28	1.41	19.5	93.0	94.1	97.7
				Mean	98.0	98.0	98.6
				Standard Deviation	4.85	5.53	0.95
				$\%RSD_r$	4.9	5.6	0.9
Overall				**Mean**	**95.7**	**101.0**	**101.3**
				Standard Deviation	**5.46**	**5.13**	**3.49**
				$\%RSD_I$	**5.7**	**5.1**	**3.5**

Table AII-16. Energy drink

Day/Analyst	Replicate	BPA Concentration (µg/L)			BPA Recovery (%)		
		0.3	1.5	20	0.3 µg/L	1.5 µg/L	20 µg/L
Day 1/ Analyst 1	1	0.36	1.47	19.9	119.7	97.9	99.3
	2	0.33	1.46	19.4	108.7	97.3	97.2
	3	0.35	1.64	20.1	118.1	109.6	100.7
				Mean	115.5	101.6	99.1
				Standard Deviation	5.94	6.93	1.76
				$\%RSD_r$	5.1	6.8	1.8
Day 2/ Analyst 2	1	0.40	1.58	19.7	134.8	105.4	98.6
	2	0.40	1.62	19.7	134.9	107.9	98.5
	3	0.38	1.60	19.8	125.9	106.7	99.0
				Mean	131.8	106.7	98.7
				Standard Deviation	5.17	1.25	0.26
				$\%RSD_r$	3.9	1.2	0.2
Day 3/ Analyst 2	1	0.39	1.52	20.5	129.5	101.5	102.4
	2	0.40	1.53	19.3	134.5	102.2	96.5
	3	0.39	1.53	20.1	131.5	101.8	100.3
				Mean	131.8	101.8	99.7
				Standard Deviation	2.52	0.35	2.99
				$\%RSD_r$	1.9	0.3	3.0
Overall				**Mean**	**126.4**	**103.4**	**99.1**
				Standard Deviation	**9.16**	**4.31**	**1.80**
				$\%RSD_i$	**7.3**	**4.2**	**1.8**

Table AII-17. Grain-based beverage

Day/Analyst	Replicate	BPA Concentration (µg/L)			BPA Recovery (%)		
		0.3	1.5	20	0.3 µg/L	1.5 µg/L	20 µg/L
Day 1/ Analyst 1	1	0.27	1.59	21.0	90.8	105.8	104.9
	2	0.31	1.40	20.3	102.1	93.4	101.5
	3	0.29	1.47	21.4	95.4	97.8	106.9
				Mean	96.1	99.0	104.4
				Standard Deviation	5.68	6.29	2.73
				$\%RSD_r$	5.9	6.4	2.6
Day 2/ Analyst 2	1	0.33	1.36	19.7	108.5	90.6	98.4
	2	0.39	1.50	20.6	128.9	100.2	103.0
	3	0.34	1.55	20.6	112.9	103.1	103.2
				Mean	116.8	98.0	101.5
				Standard Deviation	10.74	6.54	2.72
				$\%RSD_r$	9.2	6.7	2.7
Day 3/ Analyst 2	1	0.29	1.43	19.3	97.5	95.5	96.4
	2	0.29	1.44	18.4	97.1	96.1	91.8
	3	0.31	1.49	19.3	101.7	99.1	96.4
				Mean	98.7	96.9	94.8
				Standard Deviation	2.55	1.93	2.66
				$\%RSD_r$	2.6	2.0	2.8
Overall				**Mean**	**103.9**	**98.0**	**100.3**
				Standard Deviation	**11.54**	**4.73**	**4.85**
				$\%RSD_i$	**11.1**	**4.8**	**4.9**

Table AI-18. Meal replacement beverage

Day/Analyst	Replicate	BPA Concentration (µg/L)			BPA Recovery (%)		
		0.3	1.5	20	0.3 µg/L	1.5 µg/L	20 µg/L
Day 1/ Analyst 1	1	0.28	1.55	21.9	94.6	103.3	109.4
	2	0.29	1.52	20.5	97.7	101.4	102.4
	3	0.30	1.57	20.5	101.2	104.9	102.4
				Mean	97.8	103.2	104.7
				Standard Deviation	3.30	1.75	4.04
				$\%RSD_r$	3.4	1.7	3.9
Day 2/ Analyst 2	1	0.34	1.62	20.5	113.1	108.2	102.7
	2	0.36	1.54	20.2	119.1	102.7	101.0
	3	0.35	1.55	20.2	117.3	103.6	100.9
				Mean	116.5	104.8	101.5
				Standard Deviation	3.08	2.95	1.01
				$\%RSD_r$	2.7	2.8	1.0
Day 3/ Analyst 2	1	0.32	1.77	21.6	106.2	118.1	108.2
	2	0.34	1.57	20.4	113.9	105.0	102.2
	3	0.34	1.52	20.2	113.8	101.1	101.0
				Mean	111.3	108.1	103.8
				Standard Deviation	4.42	8.91	3.96
				$\%RSD_r$	4.0	8.2	3.7
Overall				**Mean**	**108.5**	**105.4**	**103.4**
				Standard Deviation	**8.92**	**5.23**	**3.18**
				$\%RSD_I$	**8.2**	**5.0**	**3.1**

APPENDIX III Accuracy and Repeatability Guidelines

Taken from "Appendix K, Guidelines for Dietary Supplements and Botanicals, AOAC Official Methods of Analysis, AOAC International, 19th edition, Gaithersburg, MD (2012)".

Acceptable recovery is a function of the concentration and the purpose of the analysis. Some acceptable recovery requirements for individual assays are as follows:

Concentration	Recovery limits, %
100%	98-101
10%	95-102
1%	92-105
0.1%	90-108
0.01%	85-110
10 µg/g (ppm)	80-115
1 µg/g (ppm)	75-120
10 µg/kg (ppb)	70-125

Taken from "Appendix K, Guidelines for Dietary Supplements and Botanicals, AOAC Official Methods of Analysis, AOAC International, 19th edition, Gaithersburg, MD (2012)".

AOAC Repeatability Guidelines	
Concentration	Repeatability (RSDR), %
100%	1
10%	1.5
1%	2
0.10%	3
0.01%	4
10 µg/g (ppm)	6
1 µg/g (ppm)	8
10 µg/kg (ppb)	15

Taken from "AOAC SMPR draft (version 7; July 11, 2017) – Determination of free Bisphenol A (BPA) in commercially packaged ready to consume carbonated and non-carbonated water and non-alcoholic beverages".

Limit of Detection (LOD)	≤ 0.1 µg/liter
Limit of Quantitation (LOQ)	≤ 0.5 µg/liter

Analytical range*	< 2 µg/liter	2 – 5 µg/liter	5 – 20 µg/liter
Accuracy	60% – 140%	80% – 120%	80% – 120%
% RSD_r	≤ 20%	≤ 10%	≤ 5%
% RSD_R	≤ 40%	≤ 20%	≤ 10%
Units are expressed as µg/liter as weight/volume. * Concentration in the ready to drink product			

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Method Validation Report Addendum

Mnemonic	BPA_LCMS
Method Title	Determination of BPA in Infant Formula, Commercial Packaged Non-Alcoholic Beverages, Pet Food and Food Packaging Migration Study Simulants by LC-MS/MS

Validation Location

- ☐ Battle Creek
- ☐ Greenfield
- ☒ Madison
- ☐ Singapore
- ☐ Other: _____

Validation Reason

- ☐ New Method
- ☐ Method Equivalency
- ☒ Method Modification
- ☐ Method Transfer
  Source Lab:_____
- ☒ Other: Matrix Extension

Protocol Identification
(if applicable) _____

DOCUMENT HISTORY:

Version #	Description	Date Issued
1	Validation on additional matrix; method reference updated	13 Jul 2018

Table of Contents

SIGNATURES

This method validation was conducted in compliance with NA-FDA 152.

This method is approved for the intended use as described in this report.

Written by: Siheng Li (1) Fell — 13JULY2018

Siheng Li
Staff Scientist
Covance Food Solutions

Date

Approved By: — 13 Jul 2018

Brent A. Rozema
Associate Director, Specialty Testing
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Date

COVANCE KEY PERSONNEL

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Technical Review	Katerina Mastovska	Associate Director

(1) signed in place of Siheng Li. MF 13JULY2018

1 INTRODUCTION

1.1 Purpose

The validation of the BPA_LCMS method was previously completed for the analysis of bisphenol A (BPA) in infant formula, beverages and food simulating liquids (used in food packaging migration studies). The purpose of this study was to extend the validation of the BPA_LCMS method for an additional matrix: pet food.

1.2 Scope

The method extension was validated in a pet food sample. The validation evaluated specificity, linearity, accuracy and precision. Due to the lack of certified reference materials, the validation study assessed accuracy and precision using spikes of the study matrix.

Study Materials

Study Matrix: Dry pet food sample with meat and vegetables
Covance ID: 7318487
Storage Condition: Room Temperature

1.3 Method

1.3.1 Method Description

The sample is extracted with water and 1% acetic acid in acetonitrile after the addition of stable-isotope labeled BPA internal standard (BPA-d16). Sodium chloride is added to salt out BPA into the acetonitrile phase. After centrifugation, a freeze-out step is used to remove co-extracted lipids. An aliquot of the supernatant upper layer is then analyzed using high-pressure liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) in electrospray negative ionization mode.

1.3.2 Method Reference

"Determination of Bisphenol A (BPA) in Commercially Packaged Ready to Consume Carbonated and Non-Carbonated Water and Non-Alcoholic Beverages", Covance developed method, accepted as AOAC First Action Official Method 2017.15

1.3.3 Supporting method references

Shi, Z., Fu, H., Xu, D. Huai, Q., Zhang, H., "Salting-Out Assisted Liquid/Liquid Extraction Coupled with Low-Temperature Purification for Analysis of Endocrine-Disrupting Chemicals in Milk and Infant Formula by Ultra High Performance Liquid Chromatography-Tandem Mass Spectrometry," *Food Analytical Methods*, 10 (5): 1523-1534 (2017)

1.3.4 Method Modification

The analysis of BPA in pet food samples required modification of the calibration range and internal standard concentration used in the MP-BPA_LCMS method. Due to the larger observed matrix effects in the pet food samples, the LOQ in pet food has been established and validated at 5 ng/g. Additionally, the less sensitive internal standard MS/MS transition is used for the BPA quantification in pet food samples.

2 MAJOR COMPUTER SYSTEMS AND INSTRUMENTATION

The major computer systems used on this study included, but not limited to, the following systems:

- MassHunter (Agilent)
- NIMS, Labware (Laboratory Information Management System)
- Agilent 6495 Mass Spectrometer

3 METHOD VALIDATION

3.1 Method Validation Procedure and Criteria

Procedure:
- Day 1: 3 replicates of validation sample and spikes at 5, 7, 10 ng/g
- Day 2: 3 replicates of validation sample and spikes at 5 ng/g
- Day 3: 3 replicates of validation sample and spikes at 5 ng/g

Linearity:
- $r^2 \geq 0.995$
- Calibration curve residuals ≤15% for the lowest level calibration standard (WS1) and ≤10% for other calibration levels

Accuracy and precision:
- Recovery of 70 – 125%
- %RSD ≤ 15%

Acceptability criteria from "Appendix K, Guidelines for Dietary Supplements and Botanicals, AOAC Official Methods of Analysis, AOAC International, 19th edition, Gaithersburg, MD (2012)".

3.2 Method Validation Results

3.2.1 Specificity

Specificity was evaluated on Days 1, 2, and 3 by analyzing method (reagent) blanks. All method blanks met the data acceptability requirements (no more than 50% reporting limit level of BPA). **Figure 1** shown in Appendix A provides representative extracted ion chromatograms of the method blank, evaluated sample matrix and the same sample matrix spiked with BPA at 5 ng/g.

Moreover, specificity was also evaluated by comparing the ion ratios for two different MS/MS qualifier ion transitions (m/z 227.0>133.0 and 227.0>93.1) in calibration standards and spikes. **Table 1** provides mean ion ratios (calculated *vs.* the quantification transition m/z 227.0>211.9) in calibration standards and spikes obtained during the validation and gives relative percent difference from the mean ion ratios obtained in the calibration standards. The relative percent differences were acceptable (well within the 30% tolerance) for both MS/MS qualifier ion transitions.

Table 1. Mean ion ratios obtained during the validation in calibration standards (*n* = 36; 6 concentration levels, 2 sets on 3 days) and pet food samples spiked at 5, 7, and 10 ng/g

	Mean ion ratio (%)*		% Relative difference**	
	m/z 227.0 > 133.0	*m/z* 227.0 > 93.1	*m/z* 227.0 > 133.0	*m/z* 227.0 -> 93.1
Calibration standards (*n* = 36)	36.0	5.5	--	--
Spikes at 5 ng/g (*n* = 9)	37.8	5.4	4.8	1.1
Spikes at 7 ng/g (*n* = 3)	34.8	5.1	3.5	5.8
Spikes at 10 ng/g (*n* = 3)	34.2	5.2	5.1	4.1

*Ion ratios calculated *vs.* the quantification transition *m/z* 227.0 > 211.9

**Relative difference compares the mean ion ratios in spikes to mean ion ratios in calibration standards, calculated based on unrounded values.

3.2.2 Range

The method accuracy and precision was evaluated for BPA in pet food from 5 ng/g to 10 ng/g, using calibration range corresponding to 3 – 100 ng/g (**Table 2**).

3.2.3 Linearity

The linearity was evaluated by analyzing calibration standards ranging from 0.45 to 15 ng/mL (corresponding to 3.0 ng/g to 100 ng/g in pet food samples) and generating calibration curves by least squares weighted (1/x) linear regression analysis on Days 1 through 3. Linearity requirements for the modified method were met as the calibration standard curves had coefficients of determination (r^2) of 0.997 to 0.999, which met the acceptance criterion of greater than or equal to 0.995. Calibration point residuals (percent difference of the back-calculated concentrations from the theoretical concentrations) were also acceptable, being within ±15% for the lowest calibration level and ±10% for the other calibration levels. See **Table 2** for the results and **Figure 2** for calibration curves.

Table 2. Linearity results - calibration point residuals for each working standard (WS) level and coefficients of determination (r^2) obtained on three different days

Description	Concentration (ng/g)	Calibration Point Residual (%)		
		Day 1	Day 2	Day 3
WS1-1	3.0	-2.4	12.9	-12.8
WS1-2	3.0	-1.9	-0.6	13.1
WS2-1	5.0	3.5	1.7	5.3
WS2-2	5.0	4.9	2.2	3.7
WS3-1	10.0	-3.2	-7.5	1.9
WS3-2	10.0	-5.7	-2.3	-7.6
WS4-1	20.0	0.2	-3.4	-1.3
WS4-2	20.0	-3.0	1.9	-0.7
WS5-1	50.0	3.7	-2.9	-0.8
WS5-2	50.0	-2.5	-8.4	-0.4
WS6-1	100.0	-1.7	6.2	-4.4
WS6-2	100.0	1.9	0.2	2.8
r^2		0.999	0.997	0.997

3.2.4 Accuracy and Precision

Accuracy (% recovery) and precision (relative standard deviation, RSD) were determined at 3 spiking levels: 5, 7, and 10 ng/g (see **Table 3** for the results). The spikes were prepared by adding an appropriate volume of the BPA spiking solution (BPA-IWS1) to the validation sample prior to extraction. For the level of 5 ng/g, accuracy, precision and intermediate precision were evaluated using the Day 1 through 3 results, with the mean recovery of 90.1% and intermediate precision RSD of 6.1% (see **Table 4** for the results) which were considered acceptable to validate 5 ng/g as the limit of quantitation (LOQ) for BPA in the tested pet food.

Table 3. Accuracy (recovery) and precision (RSD) obtained in pet food samples spiked with BPA at 5, 7, and 10 ng/g

Day	Spiking level (ng/g)	Spike sample ID	Sample Weight (g)	Spike Volume (mL)*	BPA added (ng/g)	Sample conc. (ng/g)	Mean unspiked sample conc. (ng/g)	Recovery (%)**	Mean Recovery (%)	RSD (%)
Day 1	-	7318487/4	1.504	-	-	1.36	1.38	-	N/A	N/A
		7318487/5	1.502	-	-	1.22		-		
		7318487/6	1.503	-	-	1.56		-		
	5	7388123	1.505	0.0375	5.00	6.03	-	93.0	91.0	4.4
		7388125	1.508	0.0375	4.99	5.69		86.4		
		7388127	1.506	0.0375	5.00	6.06		93.6		
	7	7388128	1.514	0.0525	6.97	7.44		86.9	89.2	3.0
		7388129	1.499	0.0525	7.03	7.61		88.6		
		7388130	1.503	0.0525	7.02	7.85		92.2		
	10	7388131	1.497	0.0750	10.1	9.98		85.1	90.2	4.9
		7388132	1.504	0.0750	10.0	10.7		93.2		
		7388133	1.506	0.0750	10.0	10.6		92.2		
Day 2	-	7318487/7	1.536	-	-	1.83	2.04	-	N/A	N/A
		7318487/8	1.525	-	-	2.17		-		
		7318487/9	1.519	-	-	2.12		-		
	5	7391693	1.528	0.0375	4.93	6.21	-	84.6	84.4	4.3
		7391694	1.500	0.0375	5.02	6.09		80.7		
		7391695	1.525	0.0375	4.94	6.38		87.9		
Day 3	-	7318487/10	1.525	-	-	2.13	2.12	-	N/A	N/A
		7318487/11	1.546	-	-	1.81		-		
		7318487/12	1.525	-	-	2.43		-		
	5	7395324	1.556	0.0375	4.84	6.84	-	97.5	95.0	2.3
		7395327	1.529	0.0375	4.93	6.73		93.5		
		7395330	1.530	0.0375	4.92	6.75		94.1		

*Volume of the BPA spiking solution BPA-IWS1 (200 ng/mL) added to the unspiked sample.
**Determined as marginal recovery (in %) after subtraction of mean BPA concentration in the unspiked sample from the concentration determined in the spiked sample and divided by the added concentration.

Table 4. Mean recovery and intermediate precision (RSD) obtained by two analysts on 3 different days in pet food sample spiked at 5 ng/g (*n* = 9)

Description	Spike sample ID	Recovery (%)*	Mean Recovery (%)	RSD (%)
Day 1/ Analyst 1	7388123	93.0	90.1	6.1
	7388125	86.4		
	7388127	93.6		
Day 2/ Analyst 2	7391693	84.6		
	7391694	80.7		
	7391695	87.9		
Day 3/ Analyst 2	7395324	97.5		
	7395327	93.5		
	7395330	94.1		

*Determined as marginal recovery (in %) after subtraction of mean BPA concentration in the unspiked sample from the concentration determined in the spiked sample and divided by the added concentration.

3.2.5 Limit of Quantitation (LOQ)

The LOQ for BPA in pet food matrix 5 ng/g. Acceptable precision and accuracy at this level was demonstrated during validation as presented in this report.

4 RAW DATA

Raw data is located in MassHunter and in the Covance Madison CFS central files.

5 CONCLUSION

The BPA_LCMS method with minor modifications has been demonstrated to have adequate specificity, linearity, accuracy, and precision for the analysis of BPA in pet food.

6 WRITTEN METHOD ASSOCIATED WITH THIS VALIDATION

Method name: BPA_LCMS
Effective date:
Version number: 2

APPENDIX A
REPRESENTATIVE CHROMATOGRAMS AND CALIBRATION CURVES

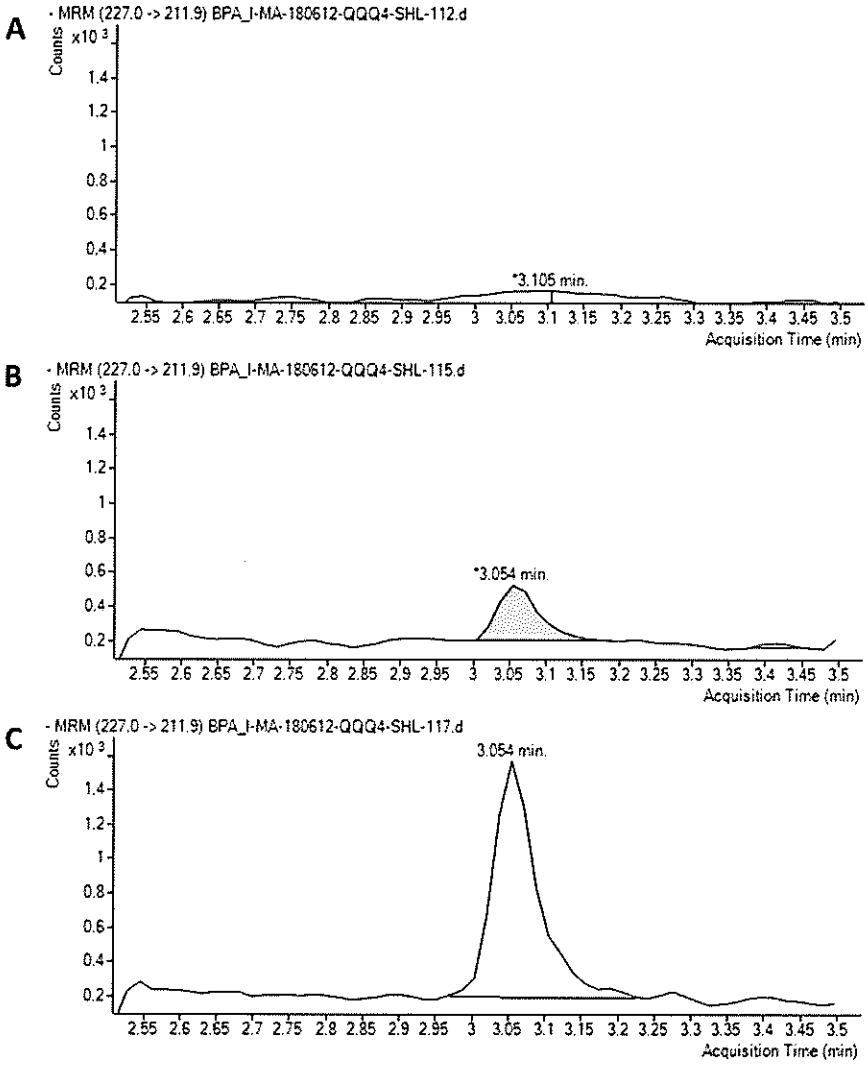


Figure 1. Representative extracted ion chromatograms of (A) method blank, (B) the evaluated sample matrix and (C) the same sample matrix spiked with BPA at 5 ng/g.

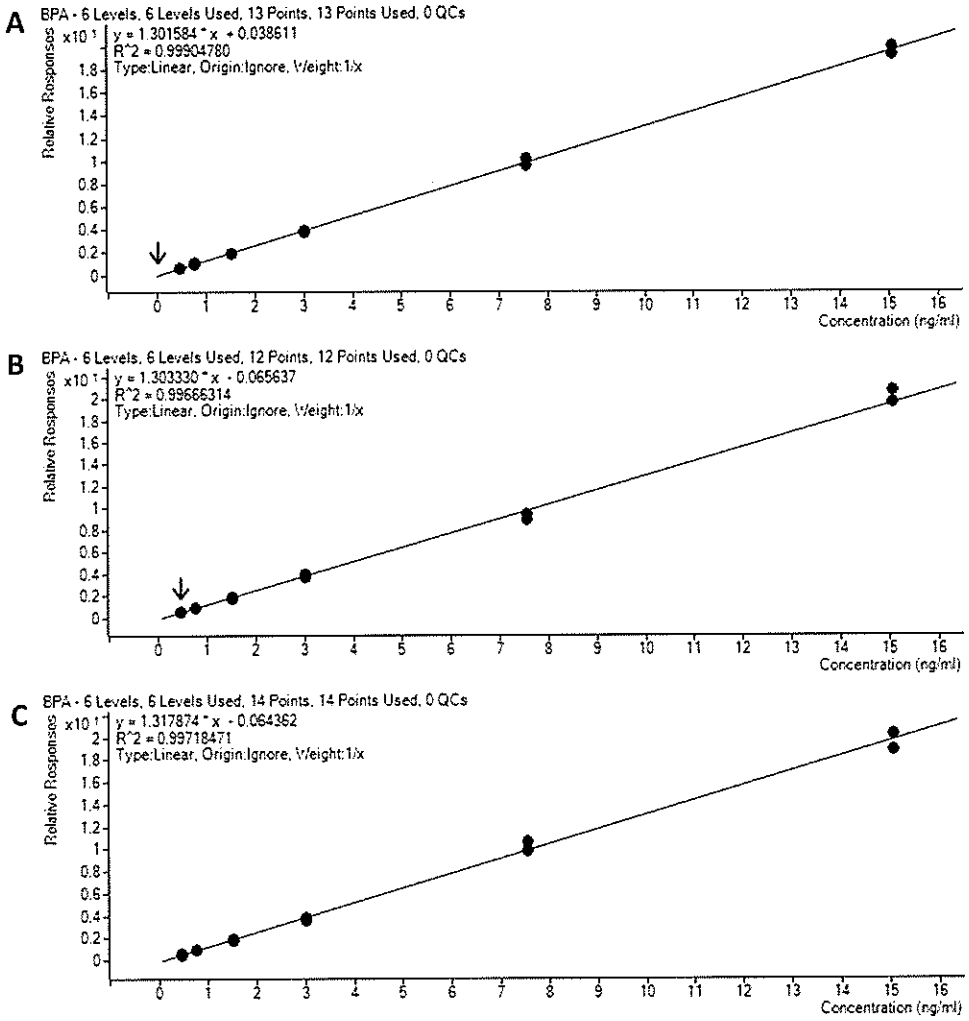


Figure 2. Calibration curves obtained on (A) Day 1, (B) Day 2 and (C) Day 3.

EXHIBIT E

UNITED STATES DISTRICT COURT
EASTERN DISTRICT OF WISCONSIN
MILWAUKEE DIVISION

SCOTT WEAVER, Individually and
on Behalf of All Others Similarly Situated,

Plaintiff,

v. Case No. 2:18-cv-1996-JPS

CHAMPION PETFOODS USA INC. and
CHAMPION PETFOODS LP,

Defendants.

**DECLARATION OF GAYAN HETTIARACHCHI
IN SUPPORT OF EXPERT REPORT OF KATERINA MASTOVSKA**

My name is Gayan Hettiarachchi and I make this declaration based on personal knowledge of the matters set out herein.

1. I am employed by Champion Petfoods LP as its Director of Food Safety. I have been employed in this role for over four years. In this role I am responsible for, among other things, overseeing food safety for both Champion Petfoods LP and Champion Petfoods USA, Inc. (collectively, "Champion").

2. During the period of May 2018 to July 2019, I, at the instruction of counsel, directed and oversaw the collection of samples of Champion's pet food products, which Champion refers to as "diets," to be shipped to an independent laboratory to be analyzed to determine the level, if any, of Bisphenol-A (BPA) present in each diet. The diets selected for analysis are those that are identified in putative class action complaints filed by plaintiffs against Champion.

3. For each of the diets, two (2) samples were collected and delivered to the independent laboratory for analysis.

4. Samples of each diet were randomly selected from existing inventory in Champion's warehouse in Auburn, Kentucky. Selected samples consisted of intact, sealed 12-ounce bags of Champion's finished dry kibble pet food and intact, sealed 16-ounce, bags of freeze-dried pet food.

5. The samples were identified by unique lot numbers or ID codes, as illustrated in the charts in the paragraphs below. Lot numbers beginning with "300" or "301" were manufactured in Champion's DogStar Kitchen, located in Auburn, Kentucky, and lot numbers beginning with "101" were manufactured in Champion's NorthStar Kitchen, located in Edmonton, Alberta, and then shipped to the DogStar Kitchen for sample collection.

6. Collection and testing of the samples were performed in four tranches. All collection of samples was performed at my direction.

7. The laboratory selected by Champion to perform this testing was Covance Food Solutions ("Covance"). After the first collection of samples was sent to Covance, but before the second, third and fourth collections of samples were sent, Covance was acquired by Eurofins Scientific ("Eurofins").

8. The first round of samples was collected by Champion and shipped to Covance's laboratory at 3301 Kinsman Boulevard, Madison, Wisconsin 53704 (the "Laboratory") on May 14, 2018. Samples of Champion diets included in this round of analysis are listed in the table below:

SAMPLE DESCRIPTION	LOT NO. OR I.D. CODE
ORIJEN Tundra Dog Food	1017750M3
ACANA Heritage Red Meat Formula Dog Food	3007599-80534
ACANA Pork and Squash Singles Formula Dog Food	3007122-73454
ORIJEN Regional Red Dog Food	3007880-80875
ACANA Wild Mackerel Singles Formula Dog Food	3008798-73204

9. The second round of samples was collected by Champion and shipped to the Laboratory on July 13, 2018. Samples of Champion diets included in this round of analysis are listed in the table below:

SAMPLE DESCRIPTION	LOT NO. OR I.D. CODE
ORIJEN Puppy Dog Food	3007509-80454
ORIJEN Six Fish Dog Food	3008011-81075
ORIJEN Regional Red Dog Food	3007826-80814
ACANA Regionals Meadowland Dog Food	3007642-80465
ACANA Regionals Appalachian Ranch Dog Food	3007252-80195
ACANA Heritage Free-Run Poultry Dog Food	3008164-81274
ACANA Heritage Freshwater Fish Dog Food	3008150-81245
ACANA Singles Lamb & Apple Dog Food	3007310-80255
ACANA Singles Duck & Pear Dog Food	3007398-80305
ACANA Singles Mackerel & Greens Dog Food	3007133-80045

10. The third round of samples was collected by Champion and shipped to Laboratory on October 4, 2018. Samples of Champion diets included in this round of analysis are listed in the table below:

SAMPLE DESCRIPTION	LOT NO. OR I.D. CODE
ORIJEN Regional Red Dog Food	1018928M3
ORIJEN Tundra Freeze-Dried Dog Food	1019167T3
ORIJEN Adult Freeze-Dried Dog Food	1018689T3
ORIJEN Six Fish Dog Food	1018435M3
ORIJEN Puppy Dog Food	1018809M3

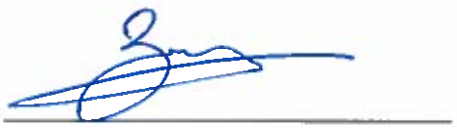
11. The fourth round of samples was collected by Champion and shipped to Laboratory on July 18, 2019. Samples of Champion diets included in this round of analysis are listed in the table below:

SAMPLE DESCRIPTION	LOT NO. OR I.D. CODE
ORIJEN Original Dog Food	3011808-91284
ORIJEN Puppy Large Breed Dog Food	3011646-91151
ORIJEN Grasslands Dog Food	3011204-90804
ORIJEN Wild Atlantic Dog Food	3011800-91274

12. It is my understanding that, upon receipt by the Laboratory of each shipment of samples, chain-of-custody documentation was initiated for all samples contained in each package.

I declare under penalty of perjury that the foregoing is true and correct.

Executed on this 2nd day of August, 2019.



Gayan Hettiarachchi

EXHIBIT F

UNITED STATES DISTRICT COURT

CENTRAL DISTRICT OF CALIFORNIA – WESTERN DIVISION

JENNIFER REITMAN, and CAROL SHOAFF individually and on behalf of a class of similarly situated individuals, Plaintiffs, v. CHAMPION PETFOODS USA, INC. and CHAMPION PETFOODS LP, Defendants.	CASE NO. 2:18-CV-01736-DOC (JPRx) Hon. David O. Carter **DECLARATION OF PAUL IRVIN**

Declaration of Paul Irvin

1. My name is Paul Irvin and I make this declaration based on personal knowledge of the matters set out herein.

2. I am a Senior Consultant at Ramboll. I am an environmental scientist with more than 10 years experience collecting samples and submitting them for laboratory analyses. I use chain of custody procedures routinely in my work collecting and documenting samples.

3. Ramboll was retained by Greenberg Traurig LLP representing Champion Petfoods and directed to obtain and submit samples of commercially available dog food products for analysis of bisphenol-A (BPA). I was assigned to obtain the products, collect the samples, prepare chain of custody documentation and ship the samples to Eurofins Food Integrity and Innovation (Eurofins) in Madison, Wisconsin.

4. I obtained packages of dog food products for BPA analysis from the routine stream of commerce, including online retail sites and retail stores in the Tampa, Florida area. The brand,

type and source for each sampled diet is identified in the Table 1, below. All packages were intact and sealed.

5. I opened each package and used a 500 ml wide-mouth jar supplied by the laboratory, Eurofins, as a scoop to collect approximately 500 ml of product.

6. I assigned a unique diet identification number (Deit ID) to each product and labeled each product package with the corresponding Diet ID. When I collected a sample of each diet, I assigned a unique sample identification number (Sample ID) and labelled the corresponding jar with this Sample ID, as documented in the photographic log attached as **Exhibit A**. The Sample IDs intentionally exclude information about the brand or type of diet in order to ensure that the laboratory was blinded to this information for each sample. I also labeled each sample jar with the corresponding Sample ID.

7. I completed the Chain of Custody form attached as **Exhibit B** documenting the collection of the samples, the request to the laboratory for analysis of BPA and the transfer of custody to the laboratory via overnight shipment. I placed all of the samples and the Chain of Custody form in a sealed cooler and dropped it off for Fedex shipping.

8. I prepared the Table 1, below, presenting the Sample IDs with the corresponding brands and types of diets for Greenberg Traurig LLP and did not transmit this information to the laboratory. I have verified that the Sample IDs shown in the table match the labeling that I applied to the packages and match the information shown on the chain of custody.

Table 1. BPA Analysis - Other Brands - Sample ID Key

Diet ID	Brand	Variety	Flavor	Source	Sample ID
D27	Fromm	Adult Dog food	Chicken and Brown Rice	Pet Wize 11401 North 56th Street Temple Terrace, FL 33617	030119-D27
D26	Farmina	N&D	Fish and Orange	Health Mutt 6116 North Central Ave Tampa, FL 33604	030119-D26
D29	Ziwi	Peak	New Zealand Tripe and Lamb	The Modern Paws 1120 East Kennedy Blvd. Unit 144 Tampa, FL 33602	030119-D29
D28	NOW (Petcurean)	NOW Fresh - Grain Free	Fish	Health Mutt 6116 North Central Ave Tampa, FL 33604	030119-D28
D2	Instinct	Original Grain Free	Beef	The Hound's Meow 16311 North Florida Ave Lutz, FL 33549	030119-D2
D30	Instinct (Duplicate)	Original Grain Free	Beef	The Hound's Meow 16311 North Florida Ave Lutz, FL 33549	030119-D30
D13	Taste of the Wild	Taste of the Wild	Pacific Stream Store # 224	Pet Supermarket 18445 US Highway 41 North Lutz, FL 33549	030119-D13
D23	Open Farm	Open Farm Freeze Dried Raw	Surf & Turf Recipe	PetFlow.com	030119-D23
D1	GO!	Fit and Free. Grain free	Chicken, Turkey, Trout	The Hound's Meow 16311 North Florida Ave Lutz, FL 33549	030119-D1
D7	Merrick	Back Country - Raw Infused	Pacific Catch - Salmon, Whitefish and Trout	Pet Supermarket Store # 224	030119-D7
D4	Blue Buffalo	Life Protection Formula	Fish and Brown Rice	Pet Supermarket Store # 224	030119-D4
--		Empty sample jar for laboratory QA.		Eurofins Madison, WI	030119-Blank

I declare under penalty of perjury that the foregoing is true and correct.

Executed on this 6 day of May, 2019.

Paul Irvin

Exhibit A

Photo 1. Diet D27. Fromm. Adult Dog food. Chicken and Brown Rice.

Photo 2: Diet D27. Fromm. Adult Dog food. Chicken and Brown Rice.

Photo 3: Sample 030119-D27. Fromm. Adult Dog food. Chicken and Brown Rice.

Photo 4: Diet D26. Farmina. N&D. Fish and Orange.

BPA Analysis – Other Brands

Sample ID Photographs

Photo 5: Sample 030119-D26. Farmina. N&D. Fish and Orange.

Photo 6: Diet D29. Ziwi. Peak. New Zealand Tripe and Lamb.

Photo 7: Sample 030119-D29. Ziwi. Peak. New Zealand Tripe and Lamb.

Photo 8: Diet D28. NOW (Petcurean). NOW Fresh - Grain Free. Fish.

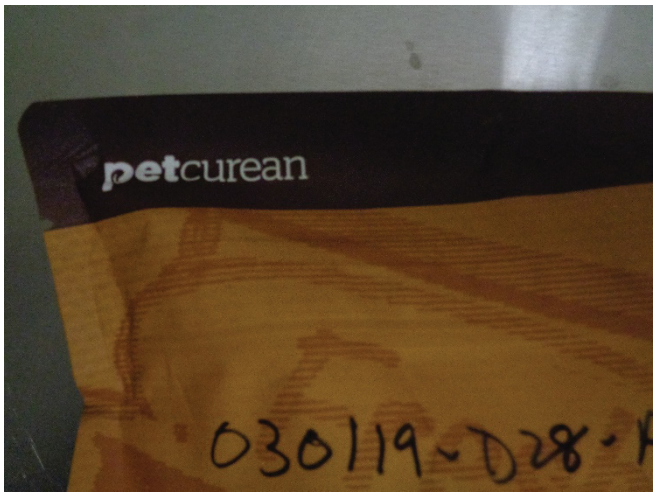


Photo 9: Diet D28. NOW (Petcurean). NOW Fresh - Grain Free. Fish.

Photo 10: Sample 030119-D28. NOW (Petcurean). NOW Fresh - Grain Free. Fish.

Photo 11: Diet D2. Instinct. Original Grain Free. Beef.

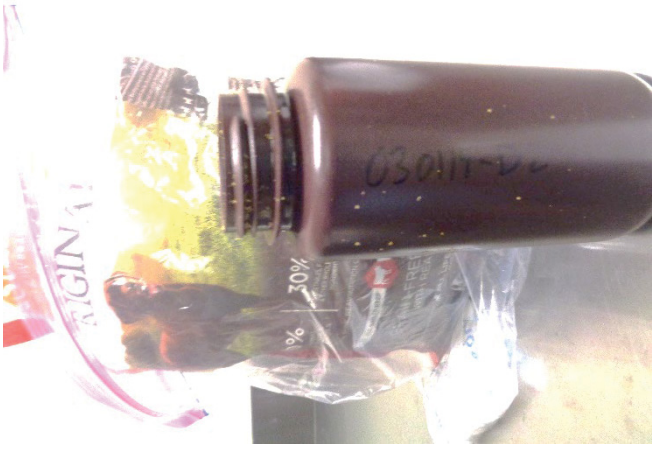


Photo 12: Sample 030119-D2. Instinct. Original Grain Free. Beef.

Photo 13: Sample 030119-D30, duplicate of Diet D2. Instinct. Original Grain Free. Beef.

Photo 14: Diet D13. Taste of the Wild. Taste of the Wild. Pacific Stream.

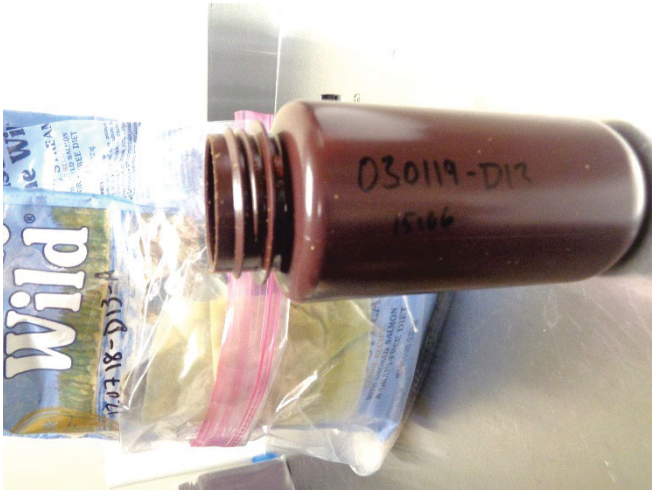


Photo 15: Sample 030119-D13. Taste of the Wild. Taste of the Wild. Pacific Stream.

Photo 16: Diet D23. Open Farm. Open Farm Freeze Dried Raw. Surf & Turf Recipe.

Photo 17: Sample 030119-D23. Open Farm. Open Farm Freeze Dried Raw. Surf & Turf Recipe.

Photo 18: Diet D1. GO! Fit and Free. Grain free. Chicken, Turkey, Trout.

Photo 19: Sample 030119-D1. GO! Fit and Free. Grain free. Chicken, Turkey, Trout.

Photo 20: Diet D7. Merrick. Back Country - Raw Infused. Pacific Catch - Salmon, Whitefish and Trout.

Photo 21: Sample 030119-D7. Merrick. Back Country - Raw Infused. Pacific Catch - Salmon, Whitefish and Trout.

Photo 22: Diet D4. Blue Buffalo. Life Protection Formula. Fish and Brown Rice.

p

Photo 23: Sample 030119-D4. Blue Buffalo. Life Protection Formula. Fish and Brown Rice.

Photo 24: Sample 030119-D4. Blue Buffalo. Life Protection Formula. Fish and Brown Rice.

Exhibit B

RAMBOLL

Chain of Custody

Page 1 of 2

Ramboll
10150 Highlands Manor Drive, Ste 440
Tampa, FL 33610
Tel: 813-628-4325 FAX: 813.628.4983

Project name: Ramboll Dogfood
Site Address
Site Name/Owner:

Date: 3/1/19

Sample ID	Date Collected	Time Collected	Description	Medium	Analysis Requested
030119-D27	3/1/19	14:21	Dry Petfood kibble	Bulk	BPA
030119-D26		14:31			
030119-D29		14:41			
030119-D28		14:52			
030119-D2		15:00			
030119-D13		15:06			
030119-D23		15:10			
030119-D1		15:14			
030119-D7		15:18			
030119-D4		15:22			

Shipped to: Eurofins Lab
Rich Schmadt
Address: 3301 Kinsman Blvd
Madison WI 53704

Shipment Method: Fed Ex
Tracking number: 7746 0109 3310
Preservation: None

Date: 3/1/19

Collected by: Name and Date: Paul Imm 3/1/19
Signature:

Comments:

Relinquished by: Name: Paul Imm
Signature: Date: 3/1/19
Time: 16:11

Received by: Name: To FedEx
Signature: Date:
Time:

Relinquished by: Name: From Fed Ex
Signature: Date:
Time:

Received by: Name:
Signature: Date:
Time:

Relinquished by: Name:
Signature: Date:
Time:

Received by: Name:
Signature: Date:
Time:

RAMBOLL

Chain of Custody

Page 2 of 2

Ramboll
10150 Highlands Manor Drive, Ste 440
Tampa, FL 33610
Tel: 813-628-4325
FAX: 813.628.4983

Project name: Ramboll Dogfood.
Site Address
Site Name/Owner:

Date: 3/1/19

Sample ID	Date Collected	Time Collected	Description	Medium	Analysis Requested
030119-D30	3/1/19	15:27	Dry petfood kibble	Bulk	BPA
030119-Blank	3/1/19	15:30			

Shipped to:

Address:

Shipment Method:

Date:

Tracking number:

Preservation:

Collected by: Name and Date:
Signature:

Comments:

Relinquished by:
Signature:
Name:
Date:
Time:

Received by:
Signature:
Name:
Date:
Time:

Relinquished by:
Signature:
Name:
Date:
Time:

Received by:
Signature:
Name:
Date:
Time:

Relinquished by:
Signature:
Name:
Date:
Time:

Received by:
Signature:
Name:
Date:
Time:

EXHIBIT G

eurofins | Food Integrity & Innovation

Report Number:	**2233043-0**
Report Date:	**06-Sep-2018**
Report Status:	**Final**

Certificate of Analysis

Greenberg Traurig, LLP

Madison Wisconsin 53704 United States

Sample Name:	**Tundra Biologically Appropriate**	**Eurofins Sample:**	**7318485**
Project ID	GREENBE_TR-20180521-0005	**Receipt Date**	16-May-2018
PO Number	Study	**Receipt Condition**	Ambient temperature
Lot Number	1017750M3	**Login Date**	21-May-2018
Sample Serving Size		**Date Started**	22-May-2018
Description	DOR4550R-12OZ	**Eurofins Study**	8390-833

Analysis	**Result**
Bisphenol A	
Bisphenol A	<5.00 ng/g
Bisphenol A	<5.00 ng/g
Bisphenol A	<5.00 ng/g

Method References	**Testing Location**
Bisphenol A (BPA_S)	**Food Integrity Innovation-Madison**

SShi, Z., Fu, H., Xu, D. Huai, Q., Zhang, H., "Salting-Out Assisted Liquid/Liquid Extraction Coupled with Low-Temperature Purification for Analysis of Endocrine-Disrupting Chemicals in Milk and Infant Formula by Ultra High Performance Liquid Chromatography-Tandem Mass Spectrometry," *Food Analytical Methods*, *10* (5): 1523-1534 (2017)

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integrity Innovation-Madison	**Edward Ladwig - Director**

Eurofins Food Chemistry Testing US, Inc.
3301 Kinsman Blvd
Madison WI 53704
800-675-8375

eurofins | Food Integrity & Innovation

Report Number:	**2233043-0**
Report Date:	**06-Sep-2018**
Report Status:	**Final**

Certificate of Analysis

Greenberg Traurig, LLP

Madison Wisconsin 53704 United States

Sample Name:	**Acana Heritage Meats Formula**	**Eurofins Sample:**	**7318486**
Project ID	GREENBE_TR-20180521-0005	**Receipt Date**	16-May-2018
PO Number	Study	**Receipt Condition**	Ambient temperature
Lot Number	3007599-80534	**Login Date**	21-May-2018
Sample Serving Size		**Date Started**	22-May-2018
Description	DAC3265-12OZ	**Eurofins Study**	8390-833

Analysis	**Result**
Bisphenol A	
Bisphenol A	<5.00 ng/g
Bisphenol A	<5.00 ng/g
Bisphenol A	<5.00 ng/g

Method References	**Testing Location**
Bisphenol A (BPA_S)	**Food Integrity Innovation-Madison**

SShi, Z., Fu, H., Xu, D. Huai, Q., Zhang, H., "Salting-Out Assisted Liquid/Liquid Extraction Coupled with Low-Temperature Purification for Analysis of Endocrine-Disrupting Chemicals in Milk and Infant Formula by Ultra High Performance Liquid Chromatography-Tandem Mass Spectrometry," *Food Analytical Methods*, *10* (5): 1523-1534 (2017)

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integrity Innovation-Madison	**Edward Ladwig - Director**

Eurofins Food Chemistry Testing US, Inc.
3301 Kinsman Blvd
Madison WI 53704
800-675-8375

eurofins | Food Integrity & Innovation

Report Number:	**2233043-0**
Report Date:	**06-Sep-2018**
Report Status:	**Final**

Certificate of Analysis

Greenberg Traurig, LLP

Madison Wisconsin 53704 United States

Sample Name:	**Acana Pork and Squash Singles Formula**	**Eurofins Sample:**	**7318487**
Project ID	GREENBE_TR-20180521-0005	**Receipt Date**	16-May-2018
PO Number	Study	**Receipt Condition**	Ambient temperature
Lot Number	3007122-73454	**Login Date**	21-May-2018
Sample Serving Size		**Date Started**	22-May-2018
Description	DAC3254-12OZ	**Eurofins Study**	8390-833

Analysis	Result
Bisphenol A	
Bisphenol A	<5.00 ng/g
Bisphenol A	<5.00 ng/g
Bisphenol A	<5.00 ng/g

Method References	Testing Location
Bisphenol A (BPA_S)	**Food Integrity Innovation-Madison**

SShi, Z., Fu, H., Xu, D. Huai, Q., Zhang, H., "Salting-Out Assisted Liquid/Liquid Extraction Coupled with Low-Temperature Purification for Analysis of Endocrine-Disrupting Chemicals in Milk and Infant Formula by Ultra High Performance Liquid Chromatography-Tandem Mass Spectrometry," *Food Analytical Methods*, *10* (5): 1523-1534 (2017)

Testing Location(s)	Released on Behalf of Eurofins by
Food Integrity Innovation-Madison	**Edward Ladwig - Director**

Eurofins Food Chemistry Testing US, Inc.
3301 Kinsman Blvd
Madison WI 53704
800-675-8375

eurofins | Food Integrity & Innovation

Report Number:	**2233043-0**
Report Date:	**06-Sep-2018**
Report Status:	**Final**

Certificate of Analysis

Greenberg Traurig, LLP

Madison Wisconsin 53704 United States

Sample Name:	**Regional Red Biologically Appropriate Dog food**	**Eurofins Sample:**	**7318488**
Project ID	GREENBE_TR-20180521-0005	**Receipt Date**	16-May-2018
PO Number	Study	**Receipt Condition**	Ambient temperature
Lot Number	3007880-80875	**Login Date**	21-May-2018
Sample Serving Size		**Date Started**	22-May-2018
Description	DOR4440-12OZ	**Eurofins Study**	8390-833

Analysis	Result
Bisphenol A	
Bisphenol A	<5.00 ng/g
Bisphenol A	<5.00 ng/g
Bisphenol A	<5.00 ng/g

Method References	**Testing Location**
Bisphenol A (BPA_S)	**Food Integrity Innovation-Madison**

SShi, Z., Fu, H., Xu, D. Huai, Q., Zhang, H., "Salting-Out Assisted Liquid/Liquid Extraction Coupled with Low-Temperature Purification for Analysis of Endocrine-Disrupting Chemicals in Milk and Infant Formula by Ultra High Performance Liquid Chromatography-Tandem Mass Spectrometry," *Food Analytical Methods*, *10* (5): 1523-1534 (2017)

Testing Location(s)	**Released on Behalf of Eurofins by**
Food Integrity Innovation-Madison	**Edward Ladwig - Director**

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Report Number:	**2233043-0**
Report Date:	**06-Sep-2018**
Report Status:	**Final**

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Madison Wisconsin 53704 United States

Sample Name:	**Acana Wild Mackerel Singles Formula**	**Eurofins Sample:**	**7318489**
Project ID	GREENBE_TR-20180521-0005	**Receipt Date**	16-May-2018
PO Number	Study	**Receipt Condition**	Ambient temperature
Lot Number	3008798-73204	**Login Date**	21-May-2018
Sample Serving Size		**Date Started**	22-May-2018
Description	DAC3257-12OZ	**Eurofins Study**	8390-833

Analysis	Result
Bisphenol A	
Bisphenol A	<5.00 ng/g
Bisphenol A	<5.00 ng/g
Bisphenol A	<5.00 ng/g

Method References	Testing Location
Bisphenol A (BPA_S)	**Food Integrity Innovation-Madison**

SShi, Z., Fu, H., Xu, D. Huai, Q., Zhang, H., "Salting-Out Assisted Liquid/Liquid Extraction Coupled with Low-Temperature Purification for Analysis of Endocrine-Disrupting Chemicals in Milk and Infant Formula by Ultra High Performance Liquid Chromatography-Tandem Mass Spectrometry," *Food Analytical Methods*, *10* (5): 1523-1534 (2017)

Testing Location(s)	Released on Behalf of Eurofins by
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Report Number:	**2478393-0**
Report Date:	**30-Apr-2019**
Report Status:	**Final**
Supercedes :	**2233044-0**

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Sample Name:	**DOR4400-12OZ / ORIJEN Puppy 12OZ**	**Eurofins Sample:**	**7501716**
Project ID	GREENBE_TR-20180719-0006	**Receipt Date**	16-Jul-2018
PO Number	Study	**Receipt Condition**	Ambient temperature
Lot Number	3007509-80454	**Login Date**	19-Jul-2018
Sample Serving Size		**Date Started**	19-Jul-2018
		Eurofins Study	LITIGATION-Greenbe_tr-BPA

Analysis	Result
Bisphenol A	
Bisphenol A	<5.00 ng/g

Method References	Testing Location
Bisphenol A (BPA_S)	**Food Integrity Innovation-Madison**
Official Methods of Analysis, Method 2017.15, AOAC INTERNATIONAL, (Modified)	

Testing Location(s)	Released on Behalf of Eurofins by
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Report Number:	**2478393-0**
Report Date:	**30-Apr-2019**
Report Status:	**Final**
Supercedes :	**2233044-0**

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Sample Name:	**DOR4430-12OZ / ORIJEN Six Fish Dog 12OZ**	**Eurofins Sample:**	**7501717**
Project ID	GREENBE_TR-20180719-0006	**Receipt Date**	16-Jul-2018
PO Number	Study	**Receipt Condition**	Ambient temperature
Lot Number	3008011-81075	**Login Date**	19-Jul-2018
Sample Serving Size		**Date Started**	19-Jul-2018
		Eurofins Study	LITIGATION-Greenbe_tr-BPA

Analysis	Result
Bisphenol A	
Bisphenol A	<5.00 ng/g

Method References	Testing Location
Bisphenol A (BPA_S)	**Food Integrity Innovation-Madison**
Official Methods of Analysis, Method 2017.15, AOAC INTERNATIONAL, (Modified)	

Testing Location(s)	Released on Behalf of Eurofins by
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Report Date:	**30-Apr-2019**
Report Status:	**Final**
Supercedes :	**2233044-0**

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Sample Name:	**DOR4440-12OZ / ORIJEN Regional Red Dog 12OZ**	**Eurofins Sample:**	**7501718**
Project ID	GREENBE_TR-20180719-0006	**Receipt Date**	16-Jul-2018
PO Number	Study	**Receipt Condition**	Ambient temperature
Lot Number	3007826-80814	**Login Date**	19-Jul-2018
Sample Serving Size		**Date Started**	19-Jul-2018
		Eurofins Study	LITIGATION-Greenbe_tr-BPA

Analysis	Result
Bisphenol A	
Bisphenol A	<5.00 ng/g

Method References	Testing Location
Bisphenol A (BPA_S)	**Food Integrity Innovation-Madison**
Official Methods of Analysis, Method 2017.15, AOAC INTERNATIONAL, (Modified)	

Testing Location(s)	Released on Behalf of Eurofins by
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Report Number:	**2478393-0**
Report Date:	**30-Apr-2019**
Report Status:	**Final**
Supercedes :	**2233044-0**

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Sample Name:	**DAC3160-12OZ / ACANA Regionals Meadowland Dog 12OZ**	**Eurofins Sample:**	**7501719**
Project ID	GREENBE_TR-20180719-0006	**Receipt Date**	16-Jul-2018
PO Number	Study	**Receipt Condition**	Ambient temperature
Lot Number	3007642-80465	**Login Date**	19-Jul-2018
Sample Serving Size		**Date Started**	19-Jul-2018
		Eurofins Study	LITIGATION-Greenbe_tr-BPA

Analysis	Result
Bisphenol A	
Bisphenol A	5.90 ng/g

Method References	Testing Location
Bisphenol A (BPA_S)	**Food Integrity Innovation-Madison**
Official Methods of Analysis, Method 2017.15, AOAC INTERNATIONAL, (Modified)	

Testing Location(s)	Released on Behalf of Eurofins by
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Report Number:	**2478393-0**
Report Date:	**30-Apr-2019**
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Sample Name:	**DAC3190-12OZ / ACANA Regionals Appalachian Ranch Dog 12OZ**	**Eurofins Sample:**	**7501720**
Project ID	GREENBE_TR-20180719-0006	**Receipt Date**	16-Jul-2018
PO Number	Study	**Receipt Condition**	Ambient temperature
Lot Number	3007252-80195	**Login Date**	19-Jul-2018
Sample Serving Size		**Date Started**	19-Jul-2018
		Eurofins Study	LITIGATION-Greenbe_tr-BPA

Analysis	Result
Bisphenol A	
Bisphenol A	<5.00 ng/g

Method References	Testing Location
Bisphenol A (BPA_S)	**Food Integrity Innovation-Madison**
Official Methods of Analysis, Method 2017.15, AOAC INTERNATIONAL, (Modified)	

Testing Location(s)	Released on Behalf of Eurofins by
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Sample Name:	**DAC3261-12OZ / ACANA Heritage Free-Run Poultry 12OZ**	**Eurofins Sample:**	**7501721**
Project ID	GREENBE_TR-20180719-0006	**Receipt Date**	16-Jul-2018
PO Number	Study	**Receipt Condition**	Ambient temperature
Lot Number	3008164-81274	**Login Date**	19-Jul-2018
Sample Serving Size		**Date Started**	19-Jul-2018
		Eurofins Study	LITIGATION-Greenbe_tr-BPA

Analysis	Result
Bisphenol A	
Bisphenol A	5.30 ng/g

Method References	Testing Location
Bisphenol A (BPA_S)	**Food Integrity Innovation-Madison**
Official Methods of Analysis, Method 2017.15, AOAC INTERNATIONAL, (Modified)	

Testing Location(s)	Released on Behalf of Eurofins by
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Supercedes :	**2233044-0**

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Sample Name:	**DAC3263-12OZ / ACANA Heritage Freshwater Fish 12OZ**	**Eurofins Sample:**	**7501722**
Project ID	GREENBE_TR-20180719-0006	**Receipt Date**	16-Jul-2018
PO Number	Study	**Receipt Condition**	Ambient temperature
Lot Number	3008150-81245	**Login Date**	19-Jul-2018
Sample Serving Size		**Date Started**	19-Jul-2018
		Eurofins Study	LITIGATION-Greenbe_tr-BPA

Analysis	Result
Bisphenol A	
Bisphenol A	19.6 ng/g

Method References	Testing Location
Bisphenol A (BPA_S)	**Food Integrity Innovation-Madison**
Official Methods of Analysis, Method 2017.15, AOAC INTERNATIONAL, (Modified)	

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Report Number:	**2478393-0**
Report Date:	**30-Apr-2019**
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Sample Name:	**DAC3251-12OZ / ACANA Singles Lamb and Apple 12OZ**	**Eurofins Sample:**	**7501723**
Project ID	GREENBE_TR-20180719-0006	**Receipt Date**	16-Jul-2018
PO Number	Study	**Receipt Condition**	Ambient temperature
Lot Number	3007310-80255	**Login Date**	19-Jul-2018
Sample Serving Size		**Date Started**	19-Jul-2018
		Eurofins Study	LITIGATION-Greenbe_tr-BPA

Analysis	Result
Bisphenol A	
Bisphenol A	<5.00 ng/g

Method References	Testing Location
Bisphenol A (BPA_S)	**Food Integrity Innovation-Madison**
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Sample Name:	**DAC3255-12OZ / ACANA Singles Duck and Pear 12OZ**	**Eurofins Sample:**	**7501724**
Project ID	GREENBE_TR-20180719-0006	**Receipt Date**	16-Jul-2018
PO Number	Study	**Receipt Condition**	Ambient temperature
Lot Number	3007398-80305	**Login Date**	19-Jul-2018
Sample Serving Size		**Date Started**	19-Jul-2018
		Eurofins Study	LITIGATION-Greenbe_tr-BPA

Analysis	Result
Bisphenol A	
Bisphenol A	<5.00 ng/g

Method References	Testing Location
Bisphenol A (BPA_S)	**Food Integrity Innovation-Madison**
Official Methods of Analysis, Method 2017.15, AOAC INTERNATIONAL, (Modified)	

Testing Location(s)	Released on Behalf of Eurofins by
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Report Number:	**2478393-0**
Report Date:	**30-Apr-2019**
Report Status:	**Final**
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Sample Name:	**DAC3257-12OZ / ACANA Singles Mackerel and Greens 12OZ**	**Eurofins Sample:**	**7501725**
Project ID	GREENBE_TR-20180719-0006	**Receipt Date**	16-Jul-2018
PO Number	Study	**Receipt Condition**	Ambient temperature
Lot Number	3007133-80045	**Login Date**	19-Jul-2018
Sample Serving Size		**Date Started**	19-Jul-2018
		Eurofins Study	LITIGATION-Greenbe_tr-BPA

Analysis	Result
Bisphenol A	
Bisphenol A	<5.00 ng/g

Method References	Testing Location
Bisphenol A (BPA_S)	**Food Integrity Innovation-Madison**
Official Methods of Analysis, Method 2017.15, AOAC INTERNATIONAL, (Modified)	

Testing Location(s)	Released on Behalf of Eurofins by
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Report Number:	**2282996-0**
Report Date:	**25-Oct-2018**
Report Status:	**Final**

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Sample Name:	**Orijen Regional Red Dog Food**	**Eurofins Sample:**	**7747568**
Project ID	GREENBE_TR-20181008-0007	**Receipt Date**	05-Oct-2018
PO Number	cvd	**Receipt Condition**	Ambient temperature
Lot Number	1018928M3	**Login Date**	08-Oct-2018
Sample Serving Size		**Date Started**	17-Oct-2018
		Eurofins Study	LITIGATION-GREENBE_TR-BPA

Analysis	Result
Bisphenol A	
Bisphenol A	<5.00 mcg/kg

Method References	Testing Location
Bisphenol A (BPA_S)	**Food Integrity Innovation-Madison**
Official Methods of Analysis, Method 2017.15, AOAC INTERNATIONAL, (Modified)	

Testing Location(s)	Released on Behalf of Eurofins by
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Report Number:	**2282996-0**
Report Date:	**25-Oct-2018**
Report Status:	**Final**

Certificate of Analysis

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Sample Name:	**Orijen Tundra Freeze Dried Dog Food**	**Eurofins Sample:**	**7747569**
Project ID	GREENBE_TR-20181008-0007	**Receipt Date**	05-Oct-2018
PO Number	cvd	**Receipt Condition**	Ambient temperature
Lot Number	1019167T3	**Login Date**	08-Oct-2018
Sample Serving Size		**Date Started**	17-Oct-2018
		Eurofins Study	LITIGATION-GREENBE_TR-BPA

Analysis	Result
Bisphenol A	
Bisphenol A	<5.00 mcg/kg

Method References	Testing Location
Bisphenol A (BPA_S)	**Food Integrity Innovation-Madison**
Official Methods of Analysis, Method 2017.15, AOAC INTERNATIONAL, (Modified)	

Testing Location(s)	Released on Behalf of Eurofins by
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Report Number:	**2282996-0**
Report Date:	**25-Oct-2018**
Report Status:	**Final**

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Sample Name:	**Orijen Adult Freeze Dried Dog Food**	**Eurofins Sample:**	**7747570**
Project ID	GREENBE_TR-20181008-0007	**Receipt Date**	05-Oct-2018
PO Number	cvd	**Receipt Condition**	Ambient temperature
Lot Number	1018689T3	**Login Date**	08-Oct-2018
Sample Serving Size		**Date Started**	17-Oct-2018
		Eurofins Study	LITIGATION-GREENBE_TR-BPA

Analysis	Result
Bisphenol A	
Bisphenol A	<5.00 mcg/kg

Method References	Testing Location
Bisphenol A (BPA_S)	**Food Integrity Innovation-Madison**
Official Methods of Analysis, Method 2017.15, AOAC INTERNATIONAL, (Modified)	

Testing Location(s)	Released on Behalf of Eurofins by
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Report Number:	**2282996-0**
Report Date:	**25-Oct-2018**
Report Status:	**Final**

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Sample Name:	**Orijen Six Fish Dog Food**	**Eurofins Sample:**	**7747571**
Project ID	GREENBE_TR-20181008-0007	**Receipt Date**	05-Oct-2018
PO Number	cvd	**Receipt Condition**	Ambient temperature
Lot Number	1018435M3	**Login Date**	08-Oct-2018
Sample Serving Size		**Date Started**	17-Oct-2018
		Eurofins Study	LITIGATION-GREENBE_TR-BPA

Analysis	Result
Bisphenol A	
Bisphenol A	<5.00 mcg/kg

Method References	Testing Location
Bisphenol A (BPA_S)	**Food Integrity Innovation-Madison**
Official Methods of Analysis, Method 2017.15, AOAC INTERNATIONAL, (Modified)	

Testing Location(s)	Released on Behalf of Eurofins by
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Report Number:	**2282996-0**
Report Date:	**25-Oct-2018**
Report Status:	**Final**

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Sample Name:	**Orijen Puppy Dog Food**	**Eurofins Sample:**	**7747572**
Project ID	GREENBE_TR-20181008-0007	**Receipt Date**	05-Oct-2018
PO Number	cvd	**Receipt Condition**	Ambient temperature
Lot Number	1018809M3	**Login Date**	08-Oct-2018
Sample Serving Size		**Date Started**	17-Oct-2018
		Eurofins Study	LITIGATION-GREENBE_TR-BPA

Analysis	Result
Bisphenol A	
Bisphenol A	<5.00 mcg/kg

Method References	Testing Location
Bisphenol A (BPA_S)	**Food Integrity Innovation-Madison**
Official Methods of Analysis, Method 2017.15, AOAC INTERNATIONAL, (Modified)	

Testing Location(s)	Released on Behalf of Eurofins by
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Report Number:	**2442612-0**
Report Date:	**28-Mar-2019**
Report Status:	**Final**

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Sample Name:	**030119-D27**	**Eurofins Sample:**	**8212745**
Project ID	GREENBE_TR-20190305-0001	**Receipt Date**	04-Mar-2019
PO Number	Study	**Receipt Condition**	Ambient temperature
Sample Serving Size		**Login Date**	05-Mar-2019
Description	3/1/19 14:21 Dry pet food Kibble Bulk	**Date Started**	20-Mar-2019
		Covance Study	LITIGATION-GREENBE_TR-BPA

Analysis	**Result**
Bisphenol A	
Bisphenol A	<5.00 ng/g

Method References	**Testing Location**
Bisphenol A (BPA_S)	**Food Integrity Innovation-Madison**
Official Methods of Analysis, Method 2017.15, AOAC INTERNATIONAL, (Modified)	

Testing Location(s)	**Released on Behalf of Eurofins by**
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Sample Name:	**030119-D26**	**Eurofins Sample:**	**8212746**
Project ID	GREENBE_TR-20190305-0001	**Receipt Date**	04-Mar-2019
PO Number	Study	**Receipt Condition**	Ambient temperature
Sample Serving Size		**Login Date**	05-Mar-2019
Description	14:31 Dry pet food Kibble Bulk	**Date Started**	20-Mar-2019
		Covance Study	LITIGATION-GREENBE_TR-BPA

Analysis	Result
Bisphenol A	
Bisphenol A	<5.00 ng/g

Method References	Testing Location
Bisphenol A (BPA_S)	**Food Integrity Innovation-Madison**
Official Methods of Analysis, Method 2017.15, AOAC INTERNATIONAL, (Modified)	

Testing Location(s)	Released on Behalf of Eurofins by
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Sample Name:	**030119-D29**	**Eurofins Sample:**	**8212747**
Project ID	GREENBE_TR-20190305-0001	**Receipt Date**	04-Mar-2019
PO Number	Study	**Receipt Condition**	Ambient temperature
Sample Serving Size		**Login Date**	05-Mar-2019
Description	14:41 Dry pet food Kibble Bulk	**Date Started**	20-Mar-2019
		Covance Study	LITIGATION-GREENBE_TR-BPA

Analysis	Result
Bisphenol A	
Bisphenol A	<5.00 ng/g

Method References	Testing Location
Bisphenol A (BPA_S)	**Food Integrity Innovation-Madison**
Official Methods of Analysis, Method 2017.15, AOAC INTERNATIONAL, (Modified)	

Testing Location(s)	Released on Behalf of Eurofins by
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Report Date:	**28-Mar-2019**
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Sample Name:	**030119-D28**	**Eurofins Sample:**	**8212748**
Project ID	GREENBE_TR-20190305-0001	**Receipt Date**	04-Mar-2019
PO Number	Study	**Receipt Condition**	Ambient temperature
Sample Serving Size		**Login Date**	05-Mar-2019
Description	14:52 Dry pet food Kibble Bulk	**Date Started**	20-Mar-2019
		Covance Study	LITIGATION-GREENBE_TR-BPA

Analysis	Result
Bisphenol A	
Bisphenol A	<5.00 ng/g

Method References	Testing Location
Bisphenol A (BPA_S)	**Food Integrity Innovation-Madison**
Official Methods of Analysis, Method 2017.15, AOAC INTERNATIONAL, (Modified)	

Testing Location(s)	Released on Behalf of Eurofins by
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Sample Name:	**030119-D2**	**Eurofins Sample:**	**8212749**
Project ID	GREENBE_TR-20190305-0001	**Receipt Date**	04-Mar-2019
PO Number	Study	**Receipt Condition**	Ambient temperature
Sample Serving Size		**Login Date**	05-Mar-2019
Description	15:00 Dry pet food Kibble Bulk	**Date Started**	20-Mar-2019
		Covance Study	LITIGATION-GREENBE_TR-BPA

Analysis	Result
Bisphenol A	
Bisphenol A	9.12 ng/g

Method References	Testing Location
Bisphenol A (BPA_S)	**Food Integrity Innovation-Madison**
Official Methods of Analysis, Method 2017.15, AOAC INTERNATIONAL, (Modified)	

Testing Location(s)	Released on Behalf of Eurofins by
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Report Number:	**2442612-0**
Report Date:	**28-Mar-2019**
Report Status:	**Final**

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Sample Name:	**030119-D13**	**Eurofins Sample:**	**8212750**
Project ID	GREENBE_TR-20190305-0001	**Receipt Date**	04-Mar-2019
PO Number	Study	**Receipt Condition**	Ambient temperature
Sample Serving Size		**Login Date**	05-Mar-2019
Description	15:06 Dry pet food Kibble Bulk	**Date Started**	20-Mar-2019
		Covance Study	LITIGATION-GREENBE_TR-BPA

Analysis	Result
Bisphenol A	
Bisphenol A	10.7 ng/g

Method References	Testing Location
Bisphenol A (BPA_S)	**Food Integrity Innovation-Madison**
Official Methods of Analysis, Method 2017.15, AOAC INTERNATIONAL, (Modified)	

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Report Status:	**Final**

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Sample Name:	**030119-D23**	**Eurofins Sample:**	**8212751**
Project ID	GREENBE_TR-20190305-0001	**Receipt Date**	04-Mar-2019
PO Number	Study	**Receipt Condition**	Ambient temperature
Sample Serving Size		**Login Date**	05-Mar-2019
Description	15:10 Dry pet food Kibble Bulk	**Date Started**	20-Mar-2019
		Covance Study	LITIGATION-GREENBE_TR-BPA

Analysis	**Result**
Bisphenol A	
Bisphenol A	<5.00 ng/g

Method References	**Testing Location**
Bisphenol A (BPA_S)	**Food Integrity Innovation-Madison**
Official Methods of Analysis, Method 2017.15, AOAC INTERNATIONAL, (Modified)	

Testing Location(s)	**Released on Behalf of Eurofins by**
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Sample Name:	**030119-D1**	**Eurofins Sample:**	**8212752**
Project ID	GREENBE_TR-20190305-0001	**Receipt Date**	04-Mar-2019
PO Number	Study	**Receipt Condition**	Ambient temperature
Sample Serving Size		**Login Date**	05-Mar-2019
Description	15:14 Dry pet food Kibble Bulk	**Date Started**	20-Mar-2019
		Covance Study	LITIGATION-GREENBE_TR-BPA

Analysis	Result
Bisphenol A	
Bisphenol A	<5.00 ng/g

Method References	Testing Location
Bisphenol A (BPA_S)	**Food Integrity Innovation-Madison**
Official Methods of Analysis, Method 2017.15, AOAC INTERNATIONAL, (Modified)	

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Sample Name:	**030119-D7**	**Eurofins Sample:**	**8212753**
Project ID	GREENBE_TR-20190305-0001	**Receipt Date**	04-Mar-2019
PO Number	Study	**Receipt Condition**	Ambient temperature
Sample Serving Size		**Login Date**	05-Mar-2019
Description	15:18 Dry pet food Kibble Bulk	**Date Started**	20-Mar-2019
		Covance Study	LITIGATION-GREENBE_TR-BPA

Analysis	Result
Bisphenol A	
Bisphenol A	8.01 ng/g

Method References	Testing Location
Bisphenol A (BPA_S)	**Food Integrity Innovation-Madison**
Official Methods of Analysis, Method 2017.15, AOAC INTERNATIONAL, (Modified)	

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Sample Name:	**030119-D30**	**Eurofins Sample:**	**8212755**
Project ID	GREENBE_TR-20190305-0001	**Receipt Date**	04-Mar-2019
PO Number	Study	**Receipt Condition**	Ambient temperature
Sample Serving Size		**Login Date**	05-Mar-2019
Description	3/1/19 15:27 Dry pet food Kibble Bulk	**Date Started**	20-Mar-2019
		Covance Study	LITIGATION-GREENBE_TR-BPA

Analysis	**Result**
Bisphenol A	
Bisphenol A	9.46 ng/g

Method References	**Testing Location**
Bisphenol A (BPA_S)	**Food Integrity Innovation-Madison**
Official Methods of Analysis, Method 2017.15, AOAC INTERNATIONAL, (Modified)	

Testing Location(s)	**Released on Behalf of Eurofins by**
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Report Number:	**2571088-0**
Report Date:	**25-Jul-2019**
Report Status:	**Final**

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Sample Name:	**Orijen Original Dog Food**	**Eurofins Sample:**	**8657388**
Project ID	GREENBE_TR-20190722-0002	**Receipt Date**	19-Jul-2019
PO Number	CVD	**Receipt Condition**	Ambient temperature
Lot Number	3011808-91284	**Login Date**	22-Jul-2019
Sample Serving Size		**Date Started**	23-Jul-2019
		Eurofins Study	LITIGATION-GREENBE_TR-BPA

Analysis	**Result**
Bisphenol A	
Bisphenol A	<5.00 ng/g

Method References	**Testing Location**
Bisphenol A (BPA_S)	**Food Integrity Innovation-Madison**
Official Methods of Analysis, Method 2017.15, AOAC INTERNATIONAL, (Modified)	

Testing Location(s)	**Released on Behalf of Eurofins by**
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Sample Name:	Orijen Puppy Large Breed Dog Food	Eurofins Sample:	8657389
Project ID	GREENBE_TR-20190722-0002	Receipt Date	19-Jul-2019
PO Number	CVD	Receipt Condition	Ambient temperature
Lot Number	3011646-91151	Login Date	22-Jul-2019
Sample Serving Size		Date Started	23-Jul-2019
		Eurofins Study	LITIGATION-GREENBE_TR-BPA

Analysis	Result
Bisphenol A	
Bisphenol A	<5.00 ng/g

Method References	Testing Location
Bisphenol A (BPA_S)	**Food Integrity Innovation-Madison**
Official Methods of Analysis, Method 2017.15, AOAC INTERNATIONAL, (Modified)	

Testing Location(s)	Released on Behalf of Eurofins by
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Sample Name:	Acana Grasslands Dog Food	Eurofins Sample:	8657390
Project ID	GREENBE_TR-20190722-0002	Receipt Date	19-Jul-2019
PO Number	CVD	Receipt Condition	Ambient temperature
Lot Number	3011204-90804	Login Date	22-Jul-2019
Sample Serving Size		Date Started	23-Jul-2019
		Eurofins Study	LITIGATION-GREENBE_TR-BPA

Analysis	Result
Bisphenol A	
Bisphenol A	5.43 ng/g

Method References	Testing Location
Bisphenol A (BPA_S)	**Food Integrity Innovation-Madison**
Official Methods of Analysis, Method 2017.15, AOAC INTERNATIONAL, (Modified)	

Testing Location(s)	Released on Behalf of Eurofins by
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Sample Name:	Acana Regionals Wild Atlantic Dog Food	Eurofins Sample:	8657391
Project ID	GREENBE_TR-20190722-0002	Receipt Date	19-Jul-2019
PO Number	CVD	Receipt Condition	Ambient temperature
Lot Number	3011800-91274	Login Date	22-Jul-2019
Sample Serving Size		Date Started	23-Jul-2019
		Eurofins Study	LITIGATION-GREENBE_TR-BPA

Analysis	Result
Bisphenol A	
Bisphenol A	<5.00 ng/g

Method References	Testing Location
Bisphenol A (BPA_S)	**Food Integrity Innovation-Madison**
Official Methods of Analysis, Method 2017.15, AOAC INTERNATIONAL, (Modified)	

Testing Location(s)	Released on Behalf of Eurofins by
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Sample Name:	Billing purposes	Eurofins Sample:	8670826
Project ID	GREENBE_TR-20190722-0002	**Receipt Date**	25-Jul-2019
PO Number	CVD	**Receipt Condition**	Ambient temperature
Sample Serving Size		**Login Date**	25-Jul-2019
		Date Started	25-Jul-2019
		Eurofins Study	LITIGATION-GREENBE_TR-BPA

Analysis	Result
Miscellaneous analysis	

Method References	Testing Location
Miscellaneous analysis (MISC_4019)	**Food Integrity Innovation-Madison**
BPA Study	

Testing Location(s)	Released on Behalf of Eurofins by
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